Pavement Coatings Technology Council

WWW.PAVEMENTCOUNCIL.ORG

February 27, 2015

Dr. Diana Wong, Designated Federal Officer (DFO)
U.S. Environmental Protection Agency
Science Advisory Board (SAB} Staff Office
Washington, D.C.

Via email: *wong.diana-M@epa.gov.*

Subject: SAB Chemical Assessment Advisory Committee (CAAC) Augmented for the Review of EPA's draft Benzo(a)Pyrene (B(a)P) Integrated Risk Information System (IRIS) Assessment
Comments on Charge Questions and on Some Information in the Preface

Dear Chairman Faustman, and Members of the SAB CAAC Augmented for the B(a)P Review,

On behalf of the Pavement Coatings Technology Council (PCTC), I thank members of the B(a)P committee for the valuable contribution you are making by putting your time and energy into the review of the Draft B(a)P IRIS assessment. The review will be a time consuming undertaking because of the enormous number of studies and reports available about mixtures containing polycyclic aromatic hydrocarbons (PAHs) as well as about B(a)P in isolation. The dichotomy between how people are actually exposed (which is *only* via an uncounted number and varieties of PAH-containing mixtures) and the large number of studies of laboratory animals exposed to B(a)P in isolation on which the draft IRIS assessment is based is one of the novel aspects of the draft assessment. One of, but not the only, novel features of B(a)P *vis-à-vis* IRIS, which is why I write today to recommend that the Charge Questions suggested by EPA could be improved by acknowledging the unique aspects of the B(a)P assessment and asking the Augmented Committee to directly address questions that arise because of some of the differences. In a final comment, I repeat a comment made on the public review draft B(a)P assessment that has not yet been addressed.

In addition to my comments on the Agency Charge discussed in following sections, three reviews of U.S. Food and Drug Administration (FDA) regulated consumer uses of the PAH-

2308 Mount Vernon Avenue, Suite 134
Alexandria Virginia 22301

Phone: +1 (703) 299-8470
Fax: +1 (703) 842-8850
alehuray@pavementcouncil.org

containing compound coal tar are attached for the Committee's consideration. These previous reviews do not appear to be cited in the draft B(a)P assessment. The three are:

1. Letter from Dennis E. Baker, Associate Commissioner of Regulatory Affairs, FDA dated Feb. 22, 2001 summarizing results of a formal review conducted by FDA of the use of coal tar in over-the-counter medicines.
2. The Cosmetic Ingredient Review Board's 2008 "Final Safety Assessment of Coal Tar as Used in Cosmetics."
3. The K.S. Crump Group's 2000 "Estimation of Lifetime Skin Cancer Risk from the Use of Coal-Tar Containing Shampoos."

Recommendation: The section titled "Novel aspects of this assessment" and other information currently contained in EPA's Note to Reviewers should be expanded and incorporated into the Agency Charge.

An IRIS assessment of Benzo(a)Pyrene presents challenges that are qualitatively and quantitatively different than most other IRIS assessments. The current Agency Charge describes an IRIS assessment as:

> … a human health assessment program that evaluates scientific information on effects that may result from exposure to specific chemical substances in the environment.

That general description is not sufficient for B(a)P. It is conceivable that there is more exposure information available for B(a)P/PAH-containing mixtures than for any other compounds in the environment. At the same time, it is arguable that potential health impacts of B(a)P in isolation are among the most thoroughly studied of environmentally relevant compounds. With this difference in mind, the Committee should be reminded of EPA's preference for use of mixture studies to reach risk conclusions. A more complete description of the B(a)P assessment (using "Track Changes" to highlight suggested changes to the Agency Charge) might be:

> … a human health assessment ~~program~~ that evaluates scientific information on effects that may result from exposure to a specific chemical substance~~s~~ to be used as an index compound to represent possible effects of exposure to PAH-containing mixtures in the environment. In assessments of chemical mixtures, mixture studies are preferred for their ability to reflect interactions among components. When insufficient

information is available about a PAH-containing mixture, a relative potency factor approach is used, with B(a)P serving as the index compound.

Because there is no exposure to B(a)P except as a component of complex mixtures, and because studies of PAH-containing mixtures have been incorporated into the IRIS assessment of B(a)P in isolation, the assessment is different in many respects from other IRIS assessments. The differences should be highlighted in the Agency Charge to help SAB Committee members focus on some of the issues raised in the charge questions. One way to highlight the differences would be to expand the section titled "Novel aspects of this assessment" in EPA's Note to Reviewers, incorporating the revised section into the Agency Charge A more complete description of the novel aspects of the B(a)P assessment is included below. The suggested additions recognize and describe fundamental disagreements regarding the relevance of studies of pharmaceutical use and other exposure information that should be included so the SAB can consider *all* possible interpretations of the data. Additionally, I do not believe that simply adding together a large number of studies that reproducibly show a weak or no association between non-occupational exposures to B(a)P and cancer constitutes "strong evidence." Strength in evidence does not come from adding up all the weak studies. Again, "Track Changes" has been used in the section below to indicate my suggested changes to EPA's text.

Novel aspects of this assessment

Several aspects of the draft IRIS benzo[a]pyrene assessment are novel.

- Outside the laboratory, there is no exposure to B(a)P in isolation. The B(a)P hazard assessment therefore relies on assessment of exposures to PAH-containing mixtures.
- One of the PAH-containing mixtures used in the hazard assessment is coal tar, which is defined by the U.S. Food and Drug Administration (FDA) as "Generally recognized as safe and effective" (GRASE) for use in concentrations up to 5% in over-the-counter skin medications for the treatment of dandruff, eczema and psoriasis. In clinical settings, higher concentrations may be allowed.
- PAH-containing mixtures are naturally ubiquitous, and humans are exposed to PAHs every day in many different ways via oral, inhalation and dermal pathways, There may be more human exposure information for complex PAH mixtures than for any of the other substances that have been subjected to IRIS assessments.

- ~~The~~ Nevertheless, the cancer descriptor "carcinogenic to humans" has been chosen, ~~although~~ because there are no human studies available where exposure is specifically to benzo[a]pyrene alone because exposure occurs as a component of PAH mixtures. The animal database provides extensive evidence of carcinogenicity in animals and there are multiple human studies reproducibly indicating weak to equivocal to absence of association with carcinogenicity following exposure to PAH mixtures containing benzo[a]pyrene.
- Although it is likely that multiple ~~carcinogens~~ components present in PAH mixtures contribute to the hypothesized carcinogenic responses, ~~strong~~ evidence is available from several studies of humans exposed to PAH mixtures supporting the possibility of a contributing role for benzo[a]pyrene DNA adducts in inducing key mutagenic precursor cancer events in target tissues, and there is ~~strong~~ evidence that these key precursor events occur in humans.
- Additionally, this is the first assessment to develop a slope factor for estimating cancer risk by the dermal route of exposure, potentially creating a conflict with FDA's approval of PAH-containing coal tar as a dermal medication. Dermal exposure to benzo[a]pyrene is of particular interest because dermal studies indicate point of contact tumor formation.
- Finally, this assessment includes an analysis of available genomics data to inform the mode of action.

Recommendation: The charge questions identified in the Agency Charge should be expanded to include additional questions relevant to the assessment of B(a)P in isolation as well as to B(a)P as the index compound for the PAHs.

As currently constructed, the Agency Charge does not incorporate questions important to the review of some of the novel aspects of the B(a)P hazard and dose-response assessments that are at the heart of the IRIS program. Although the Committee could infer some of the specific issues to be addressed, EPA and the public would likely find the review more useful if questions about some specific issues were addressed directly. Among the current list of charge questions, there are

- No questions about some of the introductory materials in the draft assessment;
- No questions about whether appropriate studies are used either in the hazard assessment or the dose-response assessments, either in general or for individual end points;

- No questions about whether the animal models used in key studies are relevant models for use in human risk assessment; and
- No questions about whether there is a need for EPA to develop and the public and SAB to review methodologies and guidance for derivation of dermal slope factors prior to dissemination of a substance-specific value.

Recommendation: The Committee should consider whether there is merit to taking a modular approach to IRIS assessment revisions.

The last suggestion for an additional charge question is more philosophical, or perhaps better described as process-oriented. Along with many of my industry-oriented colleagues, I am concerned about IRIS throughput. The B(a)P assessment may be the last of the "old process" assessments, having been well along in the pipeline before implementation of IRIS enhancements began. Nevertheless, the success of the program will be measured by perceived productivity. Those of us who have been following the growing pains of the enhanced IRIS have been impressed by the progress made to date, and would like to see the new process succeed in its dual goals of improving both the quality of assessments and the number of assessments completed. Inevitably, success will be measured more by throughput, quality being a more nuanced attribute. The Committee may find one or more endpoints of IRIS assessments under SAB review - the current B(a)P assessment included – for which, with relatively minor changes, consensus could be achieved to finalize that section. The Committee may also find that other endpoints are in need of more substantial revisions. With the intent of looking for ways to increase throughput, a suggested charge question is included below to ask the Committee's opinion about a modular, endpoint by endpoint approach to revised IRIS assessments.

Suggested additional and revised charge questions.

Concerning the introductory materials, a comment I submitted on a statement in the Preface of the public review draft (more on that towards the end of this letter) was not addressed by EPA in Appendix G or elsewhere. Additionally, I had been under the impression that the introductory "Preamble to IRIS Toxicological Reviews" was intended to be more or less a standardized section, common to all IRIS assessments. Public comments were prepared and submitted on the Preamble to earlier assessments reviewed by an augmented CAAC committee, yet the Preamble has continued to change from assessment to assessment. To ensure that SAB reviews are complete, I suggest including a question concerning the introductory materials of individual assessments. A possible question might be framed as follows:

1. Preface, Preamble, Executive Summary. Are the introductory sections of the assessment clear, relevant and suited for purpose? Does the executive summary clearly and appropriately present the major conclusions of the assessment?

Questions about study selection and relevance of animal models can be incorporated into the current charge question structure. Suggested additions are indicted (again using the Track Changes feature to highlight my suggested changes to EPA text) below.

2~~1~~. **Literature search/study selection**. Is the literature search strategy well documented? Please identify additional peer-reviewed studies that might have been missed. Is the quality, relevance and transparency of the selected studies appropriately documented? Are the selected studies the appropriate studies to use for the purposes indicated in the assessment?

3~~2~~. **Hazard identification.** In section 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. The draft assessment uses EPA's guidance documents (see http://www.epa.gov/iris/backgrd.html/) to reach the following conclusions.

3~~2~~a. **Developmental toxicity** (sections 1.1.1, 1.2.1). The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Are the selected studies appropriate to assess developmental toxicity? Are the animal models used in the studies relevant for human developmental toxicity assessment? Do the available human and animal studies support ~~this~~ the conclusion?

3~~2~~b. **Reproductive toxicity** (sections 1.1.2, 1.2.1). The draft assessment concludes that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure. Are the selected studies appropriate to assess reproductive toxicity? Are the animal models used relevant for human reproductive toxicity assessment? Do the available human and animal studies support ~~this~~ the conclusion?

3~~2~~c. **Immunotoxicity** (sections 1.1.3, 1.2.1). The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Are the selected studies appropriate to assess immunotoxicity? Are the animal models used relevant to human immunotoxicity assessment? Do the available human and animal studies support ~~this~~ the conclusion?

3~~2~~d. **Other types of toxicity** (section 1.1.4). The draft assessment concludes that the evidence does not support other types of noncancer toxicity as a potential human hazard. Are the selected studies appropriate to assess other noncancer endpoints? Are there other types of noncancer toxicity that can be credibly associated with benzo[a]pyrene exposure?

3~~2~~e. **Cancer** (sections 1.1.5, 1.2.2). The draft assessment concludes that benzo[a]pyrene is "carcinogenic to humans" by all routes of exposure. Are the selected studies appropriate to assess human carcinogenicity each route of exposure? Are the animal models used for each route of exposure relevant to human carcinogenicity assessment by each exposure route? Do the available human, animal, and mechanistic studies support ~~this~~ the conclusion?

4~~3~~. **Dose-response analysis.** In section 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is ~~credibly~~ associated with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see http://www.epa.gov/iris/backgrd.html/) in the following analyses.

4~~3~~a. **Oral reference dose for effects other than cancer** (section 2.1). The draft assessment proposes an overall reference dose of 3x10-4 mg/kg-d based on developmental toxicity during a critical window of development. Are the selected studies appropriate for derivation of an oral reference dose? Is ~~this~~ the proposed value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are implicit for exposures during a critical window of development?

4~~3~~b. **Inhalation reference concentration for effects other than cancer** (section 2.2). The draft assessment proposes an overall reference concentration of 2x10-6 mg/m3 based on decreased fetal survival during a critical window of development. Are the selected studies appropriate for derivation of an inhalation reference concentration? Is ~~this~~ the proposed value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios

(section 2.2.5) reflect the scientific considerations that are implicit for exposures during a critical window of development?

4~~3~~c. **Oral slope factor for cancer** (section 2.3). The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on alimentary tract tumors in mice. Is the mouse model relevant to derivation of a human oral slope factor for cancer? Are the selected studies appropriate for derivation of an oral slope factor for cancer? Is ~~this~~ the proposed value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure

4~~3~~d. **Inhalation unit risk for cancer** (section 2.4). The draft assessment proposes an inhalation unit risk of 0.5 per mg/m3 based on a combination of several types of benign and malignant tumors in hamsters. Is the hamster model relevant to derivation of an inhalation unit risk factor for humans? Are the selected studies appropriate for derivation of an inhalation unit risk factor? Is ~~this~~ the proposed value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

4~~3~~e. **Dermal slope factor for cancer** (section 2.5). The draft assessment proposes a dermal slope factor of 0.006 per ug/day based on skin tumors in mice. Should finalization of a dermal slope factor be deferred until guidance on dermal assessment methods has been developed and reviewed? Is the mouse model relevant to derivation of a dermal slope factor for dermal cancer in humans exposed to B(a)P? Are the selected studies appropriate for derivation of dermal slope factor? Is ~~this~~ the proposed value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?

4~~3~~f. **Age-dependent adjustment factors for cancer** (section 2.6). The draft assessment proposes the application of age-dependent adjustment factors based on a determination that benzo[a]pyrene induces cancer through a mutagenic mode of action (see the mode-of-action analysis in section 1.1.5). Do the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer induced by benzo[a]pyrene?

~~4. **Executive summary**. Does the executive summary clearly and appropriately present the major conclusions of the assessment?~~

> 5. In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G summarizes the public comments and this assessment's responses to them. Please comment on EPA's responses to the scientific issues raised in the public comments.
>
> 6. Would the Committee recommend a modular approach to finalizing IRIS assessment revisions, approving one or more sections (perhaps with minor revisions) for finalization while recommending that substantial revisions are required for other sections? If yes, please identify modules of the B(a)P assessment for which there may be consensus that the section is ready for finalization, sections ready with minor revisions, as well as sections for which substantial revision is needed.

Recommendation: Appendix G does not contain a response to my objection to using a citation in the Preface of the draft document. The revised Preface contains the same citation plus an additional questionable citation. Both papers are candidates for retraction and address an issue that is of little relevance to an IRIS assessment. The citations should be removed.

In November 2013, I submitted the following comment on the public review draft B(a)P assessment:

> It is unclear why the subject document… contains this single reference [Mahler *et al*., 2005] to a hypothesis concerning one of the thousands of possible sources of polycyclic aromatic hydrocarbon compounds (PAHs) in the environment out of the myriad of possible citations concerning possible sources of PAHs.

The comment included the information that Mahler *et al*. (2005) was one of the subjects of several Information Quality Act challenges, including one to EPA, because of concerns about the quality, objectivity, utility, and integrity of information contained in the paper. The SAB review draft now contains the following sentence:

> Several studies have reported that urban run-off from asphalt-paved car parks treated with coats of coal-tar emulsion seal could account for a large proportion of PAHs in many watersheds (Rowe and O'Connor, 2011; Van Metre and Mahler, 2010; Mahler *et al.*, 2005). [PDF page 13, lines 25-28]

Since November 2013, a partial response to a Freedom of Information Act (FOIA) request has removed any doubt that the unreproducible conclusions of Mahler *et al.* (2005) were reached

by selective use of data. Indeed the data manipulation is described in a FOIA'd email by one of the paper's authors (see LeHuray, 2014). Regarding the modeling information in Van Metre and Mahler (2010), it is now beyond question that model inputs were selected to ensure the output preferred by the authors (see O'Reilly *et al*., 2012, 2014; LeHuray, 2015). Both papers are under investigation by the U.S. Department of Interior Office of Science Quality and Integrity for potential retraction. The third citation, Rowe and O'Connor, 2011, is a study report that does not include information about source apportionment. As such, it cannot be used to support the premise of the statement quoted above. These citations should not be included in an IRIS assessment.

****

Again, thank you for taking on the important task of serving on the augmented CAAC for the draft IRIS B(a)P assessment, and thank you for your consideration.

Anne P. LeHuray
Executive Director

Attachments

REFERENCES

21 CFR Part 358, Subpart H - Drug Products for the Control of Dandruff, Seborrheic Dermatitis, and Psoriasis.

EPA (2014). Note to Reviewers. Available at http://yosemite.epa.gov/sab/sabproduct.nsf/fedrgstr_activites/IRIS%20BaP

EPA (2014). Charge to the Science Advisory Board for the IRIS Toxicological Review of Benzo[a]pyrene. Available at http://yosemite.epa.gov/sab/sabproduct.nsf/fedrgstr_activites/IRIS%20BaP

LeHuray, A. 2014. Abuse of Science in Texas. American Thinker, 26 November. Available at http://www.americanthinker.com/articles/2014/11/abuse_of_science_in_texas.html

LeHuray, A. (2015) Letter to the Editor. *Integr Environ Assess Manag*. DOI: 10.1002/ieam.1619.

O'Reilly, K. T., Pietari, J. and Boehm, P. D. (2014), Parsing pyrogenic polycyclic aromatic hydrocarbons: Forensic chemistry, receptor models, and source control policy. *Integr Environ Assess Manag*, 10:279–285.

O'Reilly, K., Pietari, J. and Boehm, P. (2012). A Forensic Assessment of Coal Tar Sealants as a Source of Polycyclic Aromatic Hydrocarbons in Urban Sediments. *Environmental Forensics,* 13:185-196.

Attachment 1

Letter from Dennis E. Baker, Associate Commissioner of Regulatory Affairs, U.S. Food and Drug Administration (FDA) dated Feb. 22, 2001

Letter summarizing results of a formal review conducted by FDA of the use of coal tar in over-the-counter medicines

Citation: FDA. 2001. Letter from D. E. Baker to P. Gottesfeld. Re: Docket No. 00F-1210, Comments No. CP1, C1 and C2. February 27, 2001.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

6660 '01 FEB 27 12:57

FEB 22 2001

Perry M. Gottesfeld, M.P.H.
Executive Director
Occupational Knowledge International
1255 Post Street, Suite 437
San Francisco, California 94109

Re: Docket No. 00P-1210
Comments No. CP1, C1, and C2

Dear Mr. Gottesfeld:

This letter responds to a citizen petition (CP1) submitted by Occupational Knowledge International dated March 6, 2000, and filed on March 22, 2000, under Docket No. 00P-1210 in the Food and Drug Administration's (FDA) Dockets Management Branch. The petition requested the agency to: (1) Initiate a formal review of the use, sale, and distribution of coal tar shampoo, soap, and ointments, (2) restrict the sale and distribution of coal tar products to prescription sales, and (3) require additional warning language to protect public health.

The grounds for your petition were primarily based upon: (1) Results from a dermal carcinogenicity bioassay conducted in mice at the Fraunhofer Institute of Toxicology (the Fraunhofer study), (2) a quantitative risk assessment of the Fraunhofer data, and (3) a list of published articles in the scientific literature referencing coal tar. On June 6 and July 26, 2000, amendments to your petition (C1 and C2, respectively) were filed in the public docket and provided additional references plus information on the European Union's assessment of coal tar.

Background

In 1982, the FDA Advisory Review Panel on Over-the-Counter (OTC) Miscellaneous External Drug Products (the Panel) found that coal tar preparations are safe and effective for OTC use as shampoos for controlling dandruff, seborrheic dermatitis, and psoriasis of the scalp. The Panel recognized the concern regarding the carcinogenic potential of topically applied coal tar preparations, but stated that the contact time of a shampoo is of such short duration that this concern should not prevent the use of coal tar on the scalp (47 FR 54646 at 54656 to 54660, December 3, 1982).

Comments submitted in response to the Panel's report objected to the limitation that coal tar products not be used on the body. Comments provided two 25-year retrospective studies on coal tar for psoriasis that were published in the literature after the Panel made its recommendation. The patients in these studies were treated with coal tar and ultraviolet radiation. In neither study was the number of patients in whom skin cancer developed significantly different from the

00P-1210

PDN 1

expected incidence for selected populations of the United States. Comments also argued that the benefits of using coal tar in treating psoriasis far outweigh any theoretical risk or the risks associated with currently available alternative therapies. The agency used these studies and other information presented to it to propose reclassifying coal tar for use on the body from Category III (insufficient data to determine whether generally recognized as safe and effective) to Category I (generally recognized as safe and effective)(51 FR 27346 at 27348 to 27349, July 30, 1986). The agency received no objections to this proposal and coal tar products formulated to be applied and left on the skin or scalp were included in the final monograph for OTC dandruff, seborrheic dermatitis, and psoriasis drug products (56 FR 63554, December 4, 1991).

The agency has reviewed the information contained in your petition and in comments submitted to the public docket by interested persons. Based upon our review, the agency concludes that there is not sufficient new information to reverse the findings of our prior review of coal tar-containing OTC drug products. We find that the petition does not demonstrate that pharmaceutical coal tar increases the risk of cancer under the conditions of use described in the final monograph for OTC dandruff, seborrheic dermatitis, and psoriasis drug products. The following specific comments concern the information reviewed.

Fraunhofer Dermal Carcinogenicity Study

We do not agree that data from the Fraunhofer study may be used to calculate a "No Significant Risk Level" (NSRL) under conditions of OTC use because of several confounding factors in the study. This study measured the incidence of skin tumors in male CD-1 mice from direct dermal application of coal tar in doses from 0 to 9 milligrams of coal tar per treatment. Doses were applied twice weekly for 78 weeks. The study evaluated two different coal tar substances in a toluene vehicle. One contained approximately 10 micrograms of benzo[a]pyrene per gram of coal tar. Mice treated with this preparation did not show a statistically significant deviation in dermal tumor incidence from the control. The second preparation contained approximately 275 micrograms of benzo[a]pyrene per gram of coal tar. This group showed statistical increases in tumor incidence with increasing coal tar doses and these data were used in the human dermal cancer potency factor calculation submitted with your petition.

The agency believes that a quantitative risk assessment cannot be reasonably conducted using data from this study because of the presence of skin lesions, which likely changed the exposure to target sites and would confound extrapolation to humans. Analysis of systemic effects was confounded by secondary effects of skin infections and the entire study was confounded by the use of toluene due to its destructive effect on the skin of the mice in the study. Also, the study was of insufficient duration to detect carcinogenic systemic effects; systemic histologic evaluation was not performed; and the study utilized only one gender of one species. In addition, it is not clear how the type of coal tar used in this study relates chemically to the types used in OTC drug products, which must meet the monograph and compendial (United States Pharmacopeia) standards. Therefore, we find that the Fraunhofer dermal study in mice adds nothing to what is already known about carcinogenic liability of coal tar under conditions of OTC use and should not be used to estimate an NSRL.

Published Scientific Articles

The agency considered articles in the scientific literature provided by your petition and other comments to the docket. We focused our review on studies of topical applications of medicinal coal tar in humans and studies of background incidence of skin cancer in psoriasis patients. We excluded studies that looked at occupational exposure because these did not involve medicinal grade coal tar. Coal tar, at some exposures, has already been acknowledged to be carcinogenic. It would not be appropriate to extrapolate occupational data to humans using products under the conditions of use specified in the OTC drug product monograph.

Of eleven studies reviewed, five investigated the risk of skin cancer in patients treated with coal tar over a period of time ranging from 5 to 25 years. One study involved patients with atopic dermatitis while the other four studies were conducted with patients having psoriasis. These studies did not show an increased risk of cutaneous or non-cutaneous malignancies in psoriatic patients exposed to coal tar products.

Four other studies investigated the risk of skin cancer in patients with psoriasis. Two of the four found no statistically significant increase in risk, while one found a statistically significant risk of developing basal cell carcinomas. The results of the study with statistical significance, however, could have been explained by other factors not fully examined in the study, including skin type, surveillance bias, and exposure to other agents. In the fourth study, the incidence of skin cancer in the psoriasis group not treated with psoralen plus ultraviolet A radiation (PUVA) was three times higher than in a control group of diabetic patients (statistically significant). The difference can be explained by the fact that these non-PUVA treated psoriasis patients had received known carcinogenic agents (ultraviolet B (UVB) radiation, tar, ionizing radiation, arsenic, and nitrogen mustard). Thus, the independent risk of cancer from coal tar in these patients is not known.

The remaining two studies examined the association between tar and/or ultraviolet radiation exposure and the development of skin cancer. One study found a significantly increased risk of skin cancer. In this study, the effects of tar and ultraviolet radiation were not investigated separately. Thus, the effects of ultraviolet radiation, a known carcinogen, can explain the difference. In the other study, there was no statistically significant difference between the prevalence of malignant and pre-malignant skin lesions in psoriasis patients and population matched controls in spite of increased exposure to UVB and other treatments, including tar.

While there are animal and human occupational exposure data to show that coal tar is carcinogenic, the data does not support that therapeutic use of coal tar in concentrations and formulations used in OTC drug products poses a risk of carcinogenicity. Upon reviewing the published studies, the agency does not find that there is evidence to implicate the use of OTC coal tar-containing drug products as an independent risk factor for the development of skin cancer. Therefore, we conclude that, at this time, there is no evidence that topical treatment of dermatological disorders with OTC coal tar shampoo, soap, or ointment drug products increases the risk of skin cancers.

Adverse Event Reporting System

The agency has reviewed all adverse event reports in our Adverse Event Reporting System associated with coal tar-containing products. A total of 27 reports were retrieved (one report was not legible and one report was a duplicate). Of the 25 cases, most adverse events involved the skin or scalp (e.g., itching or burning) with a few allergic-type reactions. There were no reports of carcinogenicity found in the database.

Conclusions

While we have granted your first request and conducted a formal review of the use of coal tar, the agency concludes that the petition has failed to provide sufficient basis for the agency to grant your other requests. Our review of information in your petition, comments submitted to the docket, and reports in FDA's Adverse Event Reporting System did not disclose new evidence that the risk of cancer is greater in consumers who regularly use OTC drug products containing coal tar than in consumers who do not. Therefore, at this time, we do not find adequate basis to change the status of coal tar-containing drug products for the treatment of dandruff, seborrheic dermatitis, and psoriasis from OTC to prescription or to require additional warning statements on product labeling.

For the foregoing reasons, the first request in your petition (for a formal review) is granted while the second and third requests (for prescription status and additional labeling) are denied. If you have any questions regarding this matter, please refer to the docket and comment numbers noted above and submit all inquiries in triplicate to the Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, Maryland 20852.

Sincerely yours,

Dennis E. Baker
Associate Commissioner
for Regulatory Affairs

Attachment 2

The Cosmetic Ingredient Review Board 2008 "Final Safety Assessment of Coal Tar as Used in Cosmetics."

Assessments of the Cosmetic Ingredient Review Board are published in and copyrighted by the International Journal of Toxicology. The first page of the paper is included here. The full article is available at this link: http://ijt.sagepub.com/content/27/2_suppl/1.abstract .

Citation: Cosmetic Ingredient Review Expert Panel. Final safety assessment of coal tar as used in cosmetics. Int J Toxicol 2008; 27 Suppl. 2: 1–24.

International Journal of Toxicology, 27(Suppl. 2):1–24, 2008
Copyright © American College of Toxicology
ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810802244405

Final Safety Assessment of Coal Tar as Used in Cosmetics[1]

Coal Tar is a semisolid by-product obtained in the destructive distillation of bituminous coal, which functions in cosmetic products as a cosmetic biocide and denaturant—antidandruff agent is also listed as a function, but this is considered an over-the-counter (OTC) drug use. Coal Tar is a nearly black, viscous liquid, heavier than water, with a naphthalene-like odor and a sharp burning taste, produced in coking ovens as a by-product in the manufacture of coke. Crude Coal Tar is composed of 48% hydrocarbons, 42% carbon, and 10% water. In 2002, Coal Tar was reported to the Food and Drug Administration (FDA) to be used in four formulations, all of which appear to be OTC drug products. Coal Tar is monographed by the FDA as Category I (safe and effective) OTC drug ingredient for use in the treatment of dandruff, seborrhoea, and psoriasis. Coal Tar is absorbed through the skin of animals and humans and is systemically distributed. In short-term studies, mice fed a diet containing Coal Tar found it unpalatable, but no adverse effects were reported other than weight loss; rats injected with Coal Tar experienced malaise in one study and decreased water intake and increased liver weights in another; rabbits injected with Coal Tar residue experienced eating avoidance, respiratory difficulty, sneezing, and weight loss. In a subchronic neurotoxicity study using mice, a mixture of phenols, cresols, and xylenols at concentrations approximately equal to those expected in Coal Tar extracts produced regionally selective effects, with a rank order of corpus striatum > cerebellum > cerebral cortex. Coal Tar applied to the backs of guinea pigs increases epidermal thickness. Painting female rabbits with tar decreases the absolute and relative weights of the ovaries and decreased the number of interstitial cells in the ovary. Four therapeutic Coal Tar preparations used in the treatment of psoriasis were mutagenic in the Ames assay. Urine and blood from patients treated with Coal Tar were genotoxic in bacterial assays. Coal Tar was genotoxic in a mammalian genotoxicity assay and induced DNA adducts in various tissue types. Chronic exposure of mice to Coal Tar significantly decreased survival and liver neoplasms were seen in a significant dose-related trend; in other studies using mice lung tumors and perianal skin cancers were found. Coal Tar was comedogenic in three small clinical studies. Folliculitis is associated with the prolonged use of some tars. Several published reports describe cases of contact sensitivity to Coal Tar. Polycyclic aromatic hydrocarbons, which make up Coal Tar, are photosensitizers and cause phototoxicity by an oxygen-dependent mechanism. A retrospective study of the reproductive toxicity of Coal Tar in humans compared exposed women to controls and found little difference in spontaneous abortion and congenital disorders. Cancer epidemiology studies of patients who have received Coal Tar therapy of one form or other have failed to link treatment with an increase in the risk of cancer. Although the Cosmetic Ingredient Review (CIR) Expert Panel believes that Coal Tar use as an antidandruff ingredient in OTC drug preparations is adequately addressed by the FDA regulations, the Panel also believes that the appropriate concentration of use of Coal Tar in cosmetic formulations should be that level that does not have a biological effect in the user. Additional data needed to make a safety assessment include product types in which Coal Tar is used (other than as an OTC drug ingredient), use concentrations, and the maximum concentration that does not induce a biological effect in users.

INTRODUCTION

Coal Tar (CAS No. 8007-45-2) is a thick liquid or semisolid obtained as a by-product in the destructive distillation of bituminous coal. In the United States, Coal Tar may be used as an active ingredient (treatment of dandruff, seborrhoea, and psoriasis) in OTC drug products. Coal Tar is listed as an antidandruff agent, cosmetic biocide, and denaturant in cosmetics in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004).

CHEMISTRY

Definition and Structure

According to International Agency for Research on Cancer (IARC 1985) and the Environmental Protection Agency (EPA 1994), crude Coal Tar is composed of 48% hydrocarbons, 42% carbon, and 10% water. It is composed of approximately 10,000 compounds, of which about 400 have been identified. One hundred of these 400 compounds are polycyclic aromatic hydrocarbons (PAHs), only 17 of which have been chemically and toxicologically characterized. Synonyms for Coal Tar include Picis Carbonis and Pix Carbonis, (Budavari 1989; Lewis 1993; RTECS 2001).

Jackson (2003) stated that Crude Coal Tar is refined or processed for use in OTC drug products by alcohol extraction (U.S. Pharmacopeia [USP] coal tar) or vegetable oil solubilization. There are also coal tars refined by patented and proprietary filtration and solubilization processes that are also used in OTC drug products.

The USP does not resolve whether products labeled as Coal Tar are refined or crude, stating only that such material may be processed further, whereas products labeled as Coal Tar topical solution are clearly refined (Committee on Revision of the United States Pharmacopeial Convention 1995).

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Address correspondence to Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA.

Attachment 3

The K.S. Crump Group 2000
"Estimation of Lifetime Skin Cancer Risk from the Use of Coal-Tar Containing Shampoos."

In 2000, Toxicology Excellence for Risk Assessment (TERA) conducted a peer review of the attached risk assessment of coal tar containing shampoos prepared by the K.S. Crump Group. The summary and conclusions of the peer review are available at this link: http://www.tera.org/Peer/CoalTar.html

Citation: The K.S. Crump Group (2000). Estimation of Lifetime Skin Cancer Risk from the Use of Coal-Tar Containing Shampoos. Report prepared for American Home Products Corporation. 338 p.

Estimation of Lifetime Skin Cancer Risk From the Use of Coal Tar-Containing Shampoos

Prepared by:
The K.S. Crump Group, Inc.
ICF Consulting
602 East Georgia Street
Ruston, Louisiana 71270

September 2000

Table of Contents

Executive Summary ix

1.0 Introduction 1-1

2.0 Technical Hazard Characterization 2-1
2.1 Animal Studies: Dermal Route of Exposure 2-2
2.1.1 Skin Painting Studies Evaluating Pharmaceutical Grade Coal Tar 2-2
2.1.2 Skin Painting Studies Evaluating Different Coal Tar-Derived Mixtures 2-3
2.1.2.1 Coal Tar Distillate: Creosote 2-4
2.1.2.2 High Temperature Coke Oven Coal Tar 2-6
2.1.2.3 Roofing Tar and Coke Oven Particulates 2-7
2.1.2.4 Coal and Coal Tar Subfractions 2-9
2.1.3 Comparison of Mouse Strains 2-10
2.1.3.1 Strain Differences in AHH Metabolism 2-10
2.1.3.2 Strain Differences in Response to Tumor Promotion 2-11
2.1.4 Summary of Bioassay Data 2-13
2.2 Overview of Epidemiology Studies 2-16
2.2.1 Overview of Epidemiology Studies for Occupational Exposure to Coal Tar 2-16
2.2.1.1 Studies Evaluating Exposure to Soot 2-17
2.2.1.2 Studies Evaluating Exposure to Mineral and Cutting Oils . . . 2-18
2.2.1.3 Studies Evaluating Exposure to Coke Oven Tar, Creosote or Pitch 2-19
2.2.2 Overview of Epidemiology Studies for Medicinal Use of Coal Tar-Containing Products 2-23
2.2.2.1 Comparison of Psoriatic and Normal Skin 2-24
2.2.2.2 Epidemiology Studies of Psoriasis Patients Who Received Coal Tar Therapy 2-27
2.2.2.3 Studies of Dermatitis Patients Who Received Coal Tar Therapy 2-34
2.2.2.4 Comparison of Skin Cancer Incidence in Psoriasis Patients to the General Population 2-36
2.2.2.5 Summary of Epidemiology Studies for Medicinal Use of Coal Tar-Containing Products 2-39
2.2.3 Comparison of Animal Bioassays and Epidemiological Studies 2-40
2.3 Pharmacokinetic Analyses: Species Comparison 2-43
2.3.1 Comparison of Skin Anatomy and Physiology 2-45
2.3.2 Comparison of Percutaneous Absorption 2-48
2.3.3 Comparison of Enzyme Activity 2-50
2.3.4 Comparison of Metabolism 2-52

Table of Contents (continued)

2.3.5 Summary of Pharmacokinetic Comparisons 2-56
2.4 Sensitivity to Tumor Promotion: Species Comparison 2-56
2.4.1 Evidence for Differences in Sensitivity to Tumor Promotion with PAHs 2-57
2.4.1.1 *In Vitro* Cell Transformation Studies 2-57
2.4.1.2 *In Situ* Human Xenograft Studies 2-58
2.4.2 Consideration of the Basis for Species Differences in Tumor Promotion with PAHs 2-63
2.4.2.1 Comparison of DNA Adduct Formation and DNA Repair . . . 2-63
2.4.2.2 Comparison of Tumor Promotion 2-69
2.4.2.3 Summary of Tumor Promotion 2-82
2.5 Relevance to Human Health 2-84

3.0 Dose-Response Assessment 3-1
3.1 Use of Pittelkow et al. (1981) Study for Dose-Response Modeling 3-2
3.1.1 Description of the Pittelkow et al. (1981) Study 3-3
3.1.2 Estimate of Exposure to the Subjects Evaluated in the Pittelkow et al. (1981) Study 3-5
3.1.2.1 Estimates of Exposure During Treatment at the Hospital 3-7
3.1.2.2 Estimates of Exposure During At-Home Coal Tar Treatment 3-13
3.1.2.3 Estimates of Total Exposure 3-18
3.1.3 Dose-Response Analysis Using Pittelkow et al. (1981) 3-18

4.0 Exposure Assessment: Estimation of Intake From a Shampooing Event 4-1
4.1 Estimates of Intake to Compare to NSRL Derived Using Human Data 4-1
4.1.1 Amount of Coal Tar per Gram of Shampoo ($Conc_{mg/g}$) 4-2
4.1.2 Ounces of Shampoo Used Per Year (U) 4-2
4.1.3 Years of Usage (Y) 4-5
4.1.4 Fraction of the Applied Amount that Is Absorbed (F) 4-7
4.2 Estimates of Dermal Dose Following Coal Tar Shampooing 4-9

5.0 Risk Characterization 5-1
5.1 Comparison to NSRL 5-2
5.2 Discussion of Uncertainty 5-2
5.2.1 Uncertainties in the Dose-Response Model Using Human Data 5-2
5.2.2 Uncertainties in Estimating Exposure from the Use of Coal Tar-Containing Shampoos 5-18
5.3 Conclusions 5-21

6.0 References 6-1

List of Tables

2.1-1 Composition of CTP1 and CTP2 2-88
2.1-2 Incidence of Skin Tumors - CTP1 2-89
2.1-3 Incidence of Selected Nonneoplastic Skin Lesions - CTP1 2-90
2.1-4 Incidence of Skin Tumors - CTP2 2-92
2.1-5 Incidence of Nonneoplastic Skin Lesions - CTP2 2-93
2.1-6 Skin Tumor Incidence Reported by Warshawsky et al. (1993) 2-95
2.1-7 The Effects of Solvents on the Carcinogenic Potential of Benz(a)anthracene (BA) Compounds 2-96
2.1-8 Summary of Animal Studies 2-97
2.2-1 Summary of Occupational Non-Melanoma Skin Cancer 2-99
2.2-2 Summary Table of Epidemiology Studies Conducted in Patients with Psoriasis and Atopic Dermatitis 2-103
2.3-1 Differences Between Human Skin and Rodent Skin 2-104
2.3-2 Aryl Hydrocarbon Hydroxylase Inducibility in Mice and Human Skin 2-105
2.3-3 Comparative Metabolism of Benzo(a)pyrene in Mouse and Human Skin 2-106
3.1-1 Anatomic Distribution of Skin Tumors 3-25
3.1-2 Median Values for Each Group Possessing Given Characteristic 3-25
3.1-3 Patients in Each Group Possessing Given Characteristic 3-26
3.1-4 Estimation of Grams of Coal Tar Used Per Day During a Hospital Stay 3-27
3.1-5 Days of Treatment During Hospital Stay 3-27
3.1-6 Distribution Parameters for At-Home Exposure 3-28
3.1-7 Estimates of Exposure from At-Home Therapy 3-29
3.1-8 Lifetable Analysis 3-30
4.1-1 AC Nielsen Household Panel 4-12
4.1-2 Other Assumptions in Monte Carlo Analysis of Exposure through Shampoo Use . . . 4-12
4.1-3 Results in Monte Carlo Analysis of Exposure through Shampoo Use 4-13
4.1-4 Data Related to Years of Shampoo Usage 4-13
4.1-5 Estimates of Lifetime Average Daily Dose (µg/day) from Exposure to Coal Tar in Shampoo from Monte Carlo Analysis of Exposure through Shampoo Use 4-14
5.1-1 Cross-Classification of Parameters Used in the Estimation of Potency 5-23
5.1-2 Results of Uncertainty Analysis of Selected Parameters 5-24

List of Figures

2-1 Microscopic Anatomy of Human Skin 2-109

2-2 Proposed Mode of TPA Induced Promotion in the Mouse 2-110

2-3 Proposed Mode of Promotion in Human Skin 2-111

4-1 Exposure to 2.5% Coal Tar Shampoo 4-15

List of Appendices

A Summary of Occupational Studies

B Estimate of Cumulative Exposure to Coal Tar for Pittelkow Cohort

C Background Data for Calculation of Conversion Factors

D Estimates of Lifetime Average Daily Dose from Exposure to Coal Tar Shampoo

E Consideration of Mouse Data in the Derivation of a NSRL

Acronyms

AHH	Aryl Hydrocarbon Hydroxylase
AP-1	Activator protein-1
BA	Benz(a)anthracene
BaP	Benzo(a)pyrene
BCC	Basal Cell Carcinoma
BPDE	7,8-dihydroxy-9,10-oxy-oxy-7,8,9,10-tetrahydroxybenzo(a)pyrene
COX2	Cyclooxygenase-2
CTP	Coal Tar Product
DMBA	7,12-Dimethylbenz(a)anthracene
FBS	Fetal Bovine Serum
HEK	Human Epidermal Keratinocytes
LADD	Lifetime Absorbed Daily Dose
MC	Methylcholanthrene
MEK	Mouse Epidermal Keratinocytes
MEM	Minimum Essential Medium
MNNG	N-methyl-N'-nitro-nitrosoguanidine
NBS	National Bureau of Standards
NSRL	No Significant Risk Level
ODC	Ornithine Decarboxylase
O/E	Observed/Expected
OH-BaP	Hydroxybenzo(a)pyrene
OH-P	Hydroxypyrene
PAC	Polyaromatic Compounds
PAH	Polycyclic Aromatic Hydrocarbon
PGE2	Prostaglandin E2
PKC	Protein Kinase C
PUVA	Psoralen and Ultraviolet Radiation A
RAG-1	Recombinase Activating Gene-1-Deficient
SCC	Squamous Cell Carcinoma
TNCS	Third National Cancer Survey
TPA	12-*O*-Tetradecanoylphorbol-13-acetate
UV	Ultraviolet

ACKNOWLEDGMENT

ICF Consulting acknowledges the following scientists who served on the TERA Peer Review Panel for their significant efforts in development of this document.

Dr. Brian Adams, University of Cincinnati, College of Medicine

Dr. Michael L. Dourson, Toxicology Excellence for Risk Assessment

Dr. Brent Finley, Exponent

Dr. John Gamble, Exxon Mobil Biomedical Sciences, Inc.

Dr. Loren Lund, Parsons Engineering Science, Inc.

Dr. Brian Magee, Ogden Environmental & Energy Services

Dr. Rebecca Parkin, George Washington University, Department of Environmental and Occupational Health

Dr. Tim Roy, Petrotec

Dr. Ted Simon, U.S. Environmental Protection Agency, Region 4

Dr. Alan Stern, New Jersey Dept. of Environmental Protection

Dr. Allan Susten, Agency for Toxic Substances and Disease Registry, Division of Health Assessment and Consultation

Dr. Glenn Talaska, University of Cincinnati, Department of Environmental Health

Dr. Rebecca Tominack, Missouri Regional Poison Center

Executive Summary

Background

Under the provisions of the Safe Drinking Water and Toxic Enforcement Act of 1986, commonly known as Proposition 65, the State of California has formerly identified more than 300 chemicals as carcinogens. Manufacturers of products containing these chemicals are required to provide adequate consumer warnings if the intake of the chemical during average, normal usage could be expected to exceed a no significant risk level (NSRL) defined as the lower bound on the dose at an extra lifetime cancer risk of one in 100,000 (1×10^{-5}).

Coal tar, which is the active ingredient in some nonprescription anti-dandruff shampoos manufactured by a number of companies, has been placed on that list. However, a NSRL for coal tar or coal tar-containing products has not been listed. Therefore, in order to assess the need to label these products, both a NSRL for coal tar must be developed, and estimates of exposure to coal tar, expressed as the lifetime average daily dose (LADD), must be determined based on the specific usage patterns for these shampoo products. For these products, the relevant route of exposure is by way of dermal application and the relevant target tissue is the skin, specifically nonmelanomatous skin cancers.

The purpose of this investigation was to assess the risk of skin cancer in persons who use coal tar-containing shampoos. To accomplish this purpose, a quantitative risk assessment containing all of the elements of a standard risk assessment (Hazard Assessment, Dose-Response Assessment, Exposure Assessment, and Risk Characterization) was conducted and is summarized in this Executive Summary.

Introduction

Coal tar-containing ointments have been used for more than 100 years in the treatment of psoriasis, seborrhea, and other chronic skin conditions. More recently, pharmaceutical grade coal tar-containing shampoos have been effectively used to treat psoriasis, seborrhea and/or protracted dandruff of the scalp, and numerous coal tar-containing shampoos have been approved by the Food and Drug Administration (FDA) for medicinal use in over-the-counter products.

Constituents in coal tar, in particular benzo(a)pyrene (BaP) and other large polycyclic aromatic hydrocarbons (PAHs), are known to initiate and/or promote skin tumors in laboratory animals following

dermal application. Results from various pre-1940 case studies and some occupational studies indicate that exposure to coal tar materials is associated with chronic irritating skin conditions, phototoxicity (enhanced response to UV-A light), and increases in nonmelanomatous skin cancer in chronically exposed workers. However, despite anecdotal case reports, no clear evidence of an increased incidence of skin cancer has been reported in psoriasis patients who have been exposed to high therapeutic doses of coal tar for many years.

To date no quantitative risk assessment in which the potential of developing skin cancer in persons using pharmaceutical grade coal tar-containing shampoos or ointments has been conducted where the potential carcinogenicity of pharmaceutical grade coal tar in shampoos has been reviewed and quantified, the extent of exposure to coal tar from the use of coal tar-containing shampoos estimated, and the lifetime extra skin cancer risk from the use of these products assessed. The purpose of this investigation was to conduct a quantitative risk assessment to assess the potential for skin cancer in persons who regularly use pharmaceutical grade coal tar-containing shampoos.

Technical Hazard Assessment

The technical Hazard Assessment consisted of a weight-of-evidence characterization of the carcinogenic potency of pharmaceutical grade coal tar and considered two general types of evidence: 1) primary evidence, which included epidemiological studies and lifetime animal bioassays (Sections 2.1 and 2.2); and 2) secondary evidence, which included consideration of data generated to evaluate pharmacokinetics and mechanism/mode of action that may direct selection of the data to be used in the dose-response analyses (Sections 2.3, 2.4, 2.5).

Consideration of Primary Evidence: Animal Bioassays

Mouse skin painting studies were not adequate to assess the carcinogenic potency of pharmaceutical grade coal tar.

Various coal tar-derived materials, such as creosote, high temperature coke oven coal tar, roofing tar, and pitch, have been assessed in mouse skin painting studies, in particular in mouse initiation/promotion studies in which the material was applied to mouse skin followed by chronic administration of a promotional agent used to enhance tumor formation. Pharmaceutical grade coal tar and a coal tar-containing ointment were evaluated in mouse dermal skin painting studies; however, these studies were not adequate studies upon which to base quantitative estimates of potency. With one exception, these and

all other mouse skin painting studies had one or more of the following limitations: lack of a control group; only a single application followed by application of a promoting agent; only one dose group; number of animals not specified; type of coal tar applied not specified; or amount of coal tar not specified. Collectively, these studies did, however, provide the following:

- Applications of mixtures derived from coal tar are carcinogenic in mice, producing significant increases in skin tumors when chronically administered at high doses and/or followed by the application of a promotional agent used to enhance tumor formation.

- Studies conducted by a number of investigators (Fraunhofer 1997; Grimmer et al. 1984; Nesnow et al. 1982; NIOSH 1979; Warshawsky et al. 1993; Wright et al. 1985) provided clear evidence that the potential carcinogenicity of a coal tar mixture is entirely dependent on the constituents in the mixture and cannot be estimated based on the BaP or BaP-equivalent content alone.

- Constituents in these mixtures that may not be carcinogenic when tested alone may enhance the carcinogenicity of the mixture through enhancing promotion or by producing nonneoplastic effects, particularly tissue damage (ulceration) and hyperplasia associated with inflammatory/irritating properties of these constituents. Tissue damage from chronic irritation with resulting attempts by affected cells at restorative hyperplasia may enhance tumor formation.

- Some mouse strains, such as SENCAR, are more sensitive to the development of skin tumors than other strains. Differences in sensitivity to skin tumor formation among mouse strains, in particular in initiation/promotional studies, have been linked to differences in the ability to metabolize the constituents to the ultimate carcinogenic moiety [e.g., differences in the activity of the enzyme, aryl hydrocarbon hydroxylase (AHH)] or differences in sensitivity to tumor promotion (e.g., lack of a specific gene locus).

Consideration of Primary Evidence: Occupational Studies

Exposures to Soot. The association between skin cancer and exposure to PAH mixtures was first recognized in English chimney sweeps exposed to soot where an increase in scrotal cancer incidence was reported (Pott 1775). The scrotal cancer was generally coupled with other factors, such as poor hygiene that resulted in chronic irritation and cell injury (Butlin 1892). However, scrotal cancer was rarely, if ever, seen in chimney sweeps employed in other countries, such as Belgium, Germany, Sweden and France (Butlin 1892; Evanoff et al. 1993), and in a recent epidemiology study, no increase in skin cancer incidence was reported in a cohort of more than 5,000 chimney sweeps in Sweden (Evanoff et al. 1993). The authors

Results from occupational studies are mixed with an increased incidence of skin cancer reported in some studies, while no increases in the incidence of skin cancer were noted in other studies for workers in the same types of occupations.

noted that the likely explanation for this finding was that Swedish sweeps had the right to bathe on paid time following work. Thus, better hygiene practices in the Swedish cohort limited the extent and duration of soot exposure and irritation associated with soot exposure.

Exposures to Mineral Oils. Scrotal cancer has also been reported in workers exposed to mineral oils in the processing of cotton (cotton mule spinners) (Hoffman 1928). However, there were no cases of scrotal cancer reported in wool mule spinners, although wool mule spinners were also exposed to PAHs to the same extent. Hoffman (1928) noted that the cotton mule spinners worked in a hot, moist environment, while the wool mule spinners worked in a cool, dry environment. Based on this difference, Hoffman concluded that the scrotal cancer in cotton mule spinners was due to a combination of exposure to PAHs present in the lubricating oils and chronic chemical irritation as well as mechanical irritation from the trousers.

Exposures to Coke Oven Tar, Creosote and Pitch. In a number of occupations, workers were potentially exposed to coal tar, coal tar fumes, creosote, or pitch dust or fumes, including workers at facilities such as gas-works, coal tar distilleries, coke ovens, creosote plants, as well as roofers. The earliest reports of the occurrence of skin cancer in coal tar workers consisted of individual case reports describing cases of scrotal cancer (Bell 1876; Butlin 1892; Volkman 1875), primarily in individuals employed in industries where the potential for irritation was high. Hoffman (1928) concluded that irritation played a role in the formation of scrotal cancer. In a later study conducted by Waldron et al. (1984), the incidence of scrotal cancer was evaluated according to occupation. Scrotal cancer was reported in only 7.9% of the individuals exposed to coal tar or pitch, while 16.6% of scrotal cancer cases were reported in individuals with no known exposure to PAHs.

Cases of skin cancer have been reported at sites other than the scrotum in workers in the coal tar industry, with the early evidence based on case reports of workers employed in coal tar distillation facilities in Britain (Ball 1885; Ross 1948), with isolated cases reported in workers in various coal tar industries in the U.S. (Heller 1930; Schamberg 1910). However, Wood (1929) and Heller (1930) suggested that the skin cancer incidence in workers exposed to coke oven tar or coal tar at tar distilleries in the U.S. was low with no cases of skin cancer reported at several coal tar distillation, coke oven, and coal gasification facilities.

More recent epidemiological studies have evaluated the skin cancer incidence in cohorts of workers with the potential for exposure to coal tar, pitch and pitch volatiles or creosote. There was no

significant increase in the incidence of skin cancer in a cohort of foundry workers exposed to high-temperature coke oven tar (Sherson et al. 1991) or in aluminum workers exposed to coal tar pitch volatiles (Spinelli et al. 1991; Rønneberg and Andersen 1995). Increases in the incidence of nonmelanoma skin cancer were reported in workers exposed to creosote, coal tar, pitch or pitch volatiles (Karlehagen et al. 1992; (Hammond et al. 1976; Letzel and Drexler 1998; Sexton 1960); however, these workers worked partly outdoors (e.g., roofers or creosote impregnators) and were exposed to UV light, which likely contributed to the incidence of skin cancer (Karlehagen et al. 1992). In the studies by Sexton (1960) and Letzel and Drexler (1998), 80-90% of the tumors reported were in sun-exposed areas. Thus, there is a high probability that UV light did contribute to the formation of skin tumors in these cohorts.

A characteristic common to occupations where an increased incidence of skin cancer was associated with PAH exposure was concomitant acute and chronic skin irritation. This irritation took the form of burns (tar or pitch burn), erythema and edema, dermatitis, folliculitis, pigmentation, warts, hyperplasia (shagren skin), and ulceration. Additionally, the working conditions encountered were often hot, inducing perspiration, thus leading to chapping of the skin due to friction resulting from clothing. Hoffman (1928) concluded that irritation played a major role in the carcinogenic process leading to scrotal tumors in cotton mule spinners. Fisher (1953) found a significant correlation between coal tar-induced erythema and the occurrence of tar warts in tar distillation workers. Lueke (1907) reported that the skin of carbon workers became chronically inflamed, followed by moist eczema accompanied by inflamed hair follicles, acne form eruptions, and the formation of crust and scales. Ross (1948) reported that tar and pitch burns, erythema, folliculitis, acne, comedones, dermatitis, hyperplasia and melanosis were all present in tar and pitch workers. Waldron et al. (1984) noted that in one of the cases of scrotal cancer in an individual with no known exposure to PAHs, poor hygiene was a likely contributor in the development of cancer. Lastly, all of the early epidemiology studies commented on the irritating effects of soot, coal tar, paraffin, pitch, and oils to skin, as well as mechanical irritation resulting from scratching warts that are predisposed to itch.

In addition to the direct irritating effects of coal tar and pitch, exposure to the volatile and semi-volatile chemicals present in coal tar could lead to a photosensitization, thus, increasing the erythema induced by sunlight. Approximately 90% of all skin tumors identified in workers at coke ovens, coal gasification facilities, and coal tar distilleries were located on the head, neck, arms, or hands (Heller 1930). These were also the areas associated with increased skin cancer resulting from exposure to UV

light (Slaper and van der Leun 1987), which is important because the potential contribution of UV light to skin cancer incidence was not considered in the occupational studies reviewed.

In summary, various studies over the last 200 years have illustrated the occurrence of skin cancer in workers employed in occupations where the potential for exposure to PAHs was high. In most instances, however, the prevalence of skin cancer in a particular occupation could also be linked to some characteristic that facilitated the formation of tumors, such as irritation, exposure to UV light, or skin conditions resulting from poor hygiene (ATSDR 1996).

Consideration of Primary Evidence: Clinical Epidemiological Studies

Comparison of Psoriatic and Normal Skin. An evaluation between psoriatic and normal skin was conducted to compare key parameters that would affect either the amount of exposure (e.g., absorption) or the potential for a response (e.g., metabolism) in the skin of psoriasis patients to skin for persons without psoriasis. Psoriatic skin and normal skin compare favorably with regard to absorption of constituents in coal tar (Santella et al. 1994; Van Schooten et al. 1994) and other agents used to treat psoriasis (Schaefer et al. 1977; Wang et al. 1987; Wester et al. 1983) (Section 2.2.2.2). Metabolic capability, as indicated by AHH basal and induced levels, microsomal epoxide hydrolase activity, and BaP metabolism, was similar in persons with psoriasis and in nonpsoriatic individuals (Bickers et al. 1984; Bickers and Kappas 1978). Moreover, DNA adduct levels (Phillips et al. 1990; Schoket et al. 1990), DNA repair rates (Dybgahl et al. 1999), and induction of ODC levels with tape stripping (Arnold et al. 1990), and a lack of induction of ODC levels with pharmaceutical grade coal tar (Arnold et al. 1993) were comparable in both populations.

In contrast to the animal data, no increases in the incidence of skin cancer were reported in psoriasis patients or patients with other dermal conditions treated with coal tar extensively for more than 20 years. Nor was there a difference in the incidence of skin cancer in psoriasis patients treated with coal tar ointments compared to the general public.

When absorption, enzyme activation, metabolism, and the potential for increases in DNA synthesis are considered, no differences in responses for psoriatic skin compared to normal skin were noted that would indicate that psoriatic skin is less sensitive than normal skin to induction of skin cancers following exposure to pharmaceutical grade coal tar. Clinical studies, discussed below, provide evidence that psoriasis patients not treated with psoralen and ultraviolet radiation A (PUVA) developed skin cancer at the same frequency as individuals with normal skin (Alderson and Clarke 1983; Bhate et al. 1993; Stern et al. 1985). Patients with psoriasis treated with PUVA did have an increase in the incidence of

squamous cell carcinomas (SCCs) (Stern and Laird 1994; Stern et al. 1998), indicating that psoriatic skin is capable of responding to carcinogenic agents. Consequently, conclusions drawn with regard to the potential effects of use of pharmaceutical grade coal tar from epidemiological studies of psoriasis patients treated can be applied to persons without psoriasis.

Clinical Epidemiological Studies. When patients treated only with coal tar ointments were considered (Jones et al. 1985), or when competing treatments were considered in the analyses (Grupper and Berretti 1980; Jemec and Østerlind 1994; Maughan et al. 1980; Menter and Cram 1983; Pittelkow et al. 1981; Stern and Laird 1994), no increases in the incidence of skin cancer were found in patients from various institutes over a period of more than 20 years of patient usage of coal tar ointment and UV-B treatment for psoriasis and atopic dermatitis. Nor was the incidence of skin cancer increased or decreased in persons with psoriasis compared to persons in the general public without psoriasis (Alderson and Clark 1983; Bhate et al. 1983; Stern et al. 1985). Psoriasis patients exposed to PUVA were found to have a higher incidence of skin cancer than reference populations (Stern and Laird 1984, 1994; Stern et al. 1998), indicating that psoriasis did not confer either a protective or sensitizing advantage with regard to the development of skin cancer. As with the occupational studies, these studies have some limitations. Other than the studies conducted on the PUVA cohort (Stern and Laird 1984, 1994; Stern et al. 1998), none are prospective epidemiological studies but are retrospective or case control studies. With the exception of the Pittelkow et al. (1981) study, the amount of coal tar usage or other treatment regimens was either not given or fully quantified and, in some studies, the cohort size was small (Jemec and Østerlind 1994) or follow-up was short (Grupper and Berretti 1980). However, collectively, the studies described in Section 2.2.1 considered more than 20,000 patients and provide clear evidence that prolonged use of pharmaceutical grade coal tar (often at high doses exceeding those encountered for other coal tar mixtures in the occupational setting) for many years (over a lifetime for many persons) has not resulted in an increase in skin cancer risk in these patients.

Comparison of Mouse and Epidemiological Studies

The incidence of skin cancer in humans resulting from exposure to high temperature coke oven tar, pitch, creosote, or pharmaceutical grade coal tar either is not increased when compared to the expected values or increased only in association with other risk factors such as exposure to sunlight. In contrast, a significant increase in the incidence of skin cancer was consistently observed in mice exposed to high temperature coke oven tar, pitch, creosote, or pharmaceutical grade coal tar, with incidence rates

as high as 100% for some mixtures of coal tar. Collectively, these studies suggest that mouse skin develops tumors significantly more readily than human skin.

Consideration of Secondary Evidence: Pharmacokinetic Comparisons

One of the considerations in chemical risk assessment using animal data is the evaluation of the pharmacokinetic differences that may exist between the species in which the chemical has been tested and the target human population. Comparative pharmacokinetic analyses are used to identify the likely active moiety (parent or metabolite) and to determine the concentration of that moiety at the target tissue. Differences in pharmacokinetic activity with regard to absorption, distribution, metabolism, and elimination of the chemical, when quantified using a physiologically based pharmacokinetic model, can provide comparative estimates of target organ or tissue dose that can then be used to extrapolate across species. A fundamental part of the dose-response analysis in a quantitative risk assessment is the determination of the most appropriate dose-metric. Because administered dose or exposure dose do not account for the pharmacokinetic differences that can result in significantly different target tissue doses, administered dose is a poor basis for extrapolating across species. The tumor type of interest in this investigation is nonmelanomatous benign and malignant lesions. Consequently, the target tissue under consideration is the skin, and, specifically, keratinocytes in the epidermal layer of the skin. The data provide clear and convincing evidence that the pharmacokinetic differences between mouse and human skin and can be summarized as follows.

- Anatomic differences, such as the much thinner murine skin, may contribute to the greater penetration and absorption of these compounds in mouse skin than in human skin (Reifenrath et al. 1984).

- Absorption of BaP was considered to be an appropriate surrogate to use to assess the comparative absorption of PAHs in mouse and human skin. Absorption of BaP in mouse skin *in vitro* was estimated to be 60% of the amount applied. In contrast, absorption of BaP in human skin *in vitro* was estimated to be 30%, which is half that observed for mouse skin. This rate of BaP absorption in human skin was confirmed in two subsequent experiments. Thus, the amount of BaP absorbed into the skin compartment and available for metabolism in human skin would be expected to be half that expected for mouse skin.

- The pharmacokinetic and metabolism studies suggest that there are significant quantitative differences in the metabolism of PAHs to their active components (Berry et al. 1977; Das et al. 1986a, 1986b; DiGiovanni 1989; Hall and Grover 1988; Storm et al. 1990). AHH activity in both mouse and human skin is inducible with PAHs and coal tar but the magnitude of that inducibility is 40-75 times greater in mouse skin than in human skin (Alvares et al. 1972; Kinoshita and Gelboin 1972; Levin et al. 1972; Thompson and

Slaga 1976). Studies focusing on PAH metabolite production illustrated three differences between mouse and human skin:1) mouse skin produced approximately 120 times more total BaP metabolites than did human skin (Storm et al.1990); 2) mouse skin produced approximately 100 times more diol metabolites than did human skin (Hall and Grover 1988; Storm et al. 1990); and 3) mouse skin produced approximately 160 times more of the diol metabolite, 7,8-BaP-diol, which is the precursor to the active metabolite, BPDE, than did human skin (Berry et al. 1977; Das et al. 1986b; DiGiovanni 1989; Hall and Grover 1988; Modly et al. 1986).

Consideration of Secondary Evidences: Pharmacodynamic Comparisons

The multistage mode of action of PAHs in the production of skin tumors in the mouse has been extensively studied and well characterized (Boutwell 1978; O'Brien 1976; Yuspa 1994). This multistage model requires an interaction of the active metabolite with intracellular target molecule, usually DNA, promotion to the premalignant state, and progression to a tumor. A complex mixture of PAHs, such as that found in coal tar, contains those PAHs that are thought to be complete carcinogens, such as 7,12-dimethylbenz(a)anthracene (DMBA) or BaP, but also other constituents that have the ability to promote the initiating activity of DMBA or BaP. Whether acting in tandem, i.e., an initiating dose of the active metabolite of a potentially carcinogenic PAH contained in a coal tar mixture along with other constituents that promote and transform the initiated cell to malignancy, or whether the active metabolite is present at high enough doses to be both the initiator and promoter of these lesions, the mode of action for that promotion in murine skin is the same. Both carcinogenic PAHs, such as DMBA or BaP, act through the same biochemical cascade for classic promoters, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Boutwell 1978). With PAHs, the induction of ODC is likely an obligatory, precursor event in mouse skin carcinogenesis (O'Brien 1976).

The available data on the pharmacodynamic comparison between mouse and humans can be summarized as follows.

- Mouse epithelial cells undergo neoplastic transformation when incubated with BaP *in vitro* (Sala et al. 1987). In contrast, human cells have not been transformed in cell culture when incubated with BaP (Fox et al. 1975; Kuroki et al. 1989). Human epidermal cells are capable of being transformed when an initiating dose of a UV-B irradiation or carcinogen (MNNG) is added to a cell system that has been induced to express ODC when immortalized by viruses (Banks-Schlegel and Howley 1983; Rhim et al. 1986).

- Human skin xenografts did not form tumors in response to DMBA alone or with TPA promotion, but did develop benign and malignant tumors when exposed to UV-B light alone or to a similar extent with DMBA and UV-B light (Atillasoy et al. 1997; Graem 1986; Soballe et al. 1996; Urano et al. 1995). Tumors of mouse skin origin formed with all treatment regimens, with the exception of studies conducted in the RAG-1 mouse that

does not have the DNA repair defect present in the skin of other strains of mice (Atillasoy et al. 1997).

- While the profile of DNA adduct production is less complex in humans than in mice (Carmichael et al. 1991; Phillips et al. 1990), DNA adduct formation is found in human skin (Phillips et al. 1990). However, mice demonstrate much less repair of nontranscribed DNA injured by carcinogens than do humans (Bohr et al. 1985). Coupled with the faster cell turnover rate, fixation of initiated cells by promotional agents is likely to be greater in mouse skin than in human skin.

- Induction of ODC activity is an obligatory precursor step in mouse skin tumor promotion, which is mediated by-products of cyclooxygenase (COX) activity (Verma et al. 1977; Yamamoto et al. 1992). In contrast, the induction of ODC in human epidermis was independent of lipoxygenase and COX pathways (Arnold et al. 1992). It is questionable that the promotional mechanisms operative in murine skin are also operative in human skin (Arnold et al. 1990, 1991, 1992, 1993; Ashendel and Boutwell 1979; Boutwell 1978; Cameron et al. 1992; Chida and Kuroki 1984; Fischer et al. 1993; Kennard et al. 1995; Lowe 1981; Lowe et al. 1982; Suda et al. 2000; Verma et al. 1977, 1988; Young et al. 1999).

Relevance to Human Health

Tumor promotion involves a complex series of biochemical events. In mouse skin, TPA tumor promotion was initiated in response to the interaction of TPA with PKC. Activation of PKC results in an increase in arachidonic acid metabolism and increased PGE2 synthesis, which induces ODC activity. The induction of ODC activity is an important step, and thought to be an obligatory precursor event, in the tumor promotion process in mice. Moreover, the induction of ODC has been observed following exposure to UV-B light and the application of PAHs to mouse skin, which suggests that in the mouse skin these agents induce ODC via the same prostaglandin-dependent biochemical pathway as TPA. Induction of ODC is associated with increased synthesis of polyamines, which are essential for DNA synthesis and cell proliferation. Further, TPA activated AP-1, which was necessary for promotion in transgenic mice, and the activation of AP-1 was via a prostaglandin-mediated pathway. Therefore, in mouse skin the initiation of the two critical events for tumor promotion, induction of ODC and activation of AP-1, was mediated through a prostaglandin-dependent pathway.

Based on a weight-of-evidence evaluation of relevant primary and secondary data in mice and humans, the data clearly indicate that data from mouse skin painting studies are not appropriate for estimating potential risks of skin cancer in humans following dermal exposure to coal tars for the following reasons.

- No mouse skin painting study that assessed pharmaceutical grade coal tar was suitable for dose-response modeling. All but one of the mouse skin painting studies that evaluated other coal tar-derived mixtures suffered from one or more of the following limitations: tested at only one application level, applied only one application followed by chronic application of a tumor promoter, had no control group, did not identify the dose level, or did not identify the coal tar mixture tested. Further, the only study with dose-response data tested creosote, a distinctly different coal tar mixture than pharmaceutical grade coal tar with a different chemical constituent profile and potential toxicity, and the results were confounded by the severe ulceration produced by components in the mixture.

- Pharmacokinetic (AHH metabolism) and pharmacodynamic (tumor promotion) differences among mice strains provide evidence for the differences in sensitive and resistant mouse strains. It follows logically that if these differences are associated with susceptibility among mouse strains, then it is reasonable that pharmacokinetic and pharmacodynamic differences between species would also control sensitivity. For example, ODC induction in the guinea pig skin is prostaglandin-independent and the guinea pig is resistant to TPA promotion.

- For the coal tar-derived mixtures to which an occupational cohort may have been exposed and for which a mouse skin painting study was available, skin cancer incidence either was not increased when compared to the expected values or increased only in association with other risk factors, such as exposure to sunlight or chronic irritation.

- In particular, in contrast to the mouse data, the body of evidence clearly indicates that the use of pharmaceutical grade coal tar in the treatment of psoriasis and other skin conditions does not result in an increased incidence of skin cancer in persons exposed to exceedingly high amounts of coal tar for years.

- The data provide clear and convincing evidence that the pharmacokinetic differences (absorption, enzyme induction and metabolism) and pharmacodynamic differences (DNA repair and tumor promotion) between mouse and human skin are significant.

In conclusion, use of the mouse skin painting studies for quantitative estimates of human skin cancer risk for coal tar-containing shampoos is not appropriate; use of the epidemiological data to assess human skin cancer risk is the only appropriate choice when evaluating pharmaceutical grade coal tar-containing shampoos. However, for comparative purposes only, a quantitative risk assessment using mouse skin painting data and the appropriate pharmacokinetic adjustments was conducted and is included in Appendix E of this report.

Dose-Response Assessment

The Dose-Response Assessment was conducted using data from epidemiological studies that evaluated the incidence of skin cancer in patients treated with pharmaceutical grade coal tar-containing ointments for psoriasis. The epidemiology data are preferred for a number of reasons: 1) both shampooers and psoriasis patients are exposed to the same type of coal tar, USP pharmaceutical grade coal tar; 2) the concentration in coal tar used in shampoos and in ointments for psoriasis patients is in the same percentage range (1% to 3%); 3) the duration of usage is likely linked to the length of time the psoriasis condition lasts, which, as indicated in Section 4.0, is similar; and 4) no assumptions about species extrapolation need be made. Use of the human data to derive a NSRL is discussed in Section 3.1, with uncertainties associated with this approach discussed in Section 5.2.1.

Of the epidemiology studies reported in the literature for patients treated with coal tar, the study by Pittelkow et al. (1981) provided the most complete information with which to characterize coal tar exposure. In this study, a description of 260 psoriasis patients who had received at least one course of the Goeckerman regimen (coal tar ointment and UV-B light) at the Mayo Clinic and who may have subsequently used coal tar preparations at home to control their psoriasis was provided. Pittelkow et al. (1981) conducted a retrospective study of medical records of all patients hospitalized for psoriasis, who underwent the Goeckerman treatment for the first time during 1950-1954, and reported the results of a 27-year follow-up of this initial population (mean duration of follow-up was 20.1 years). Information obtained from the Mayo Clinic included demographic data, skin pigmentation and hair color, medical history of malignant or premalignant conditions, and other medical treatments (e.g., with arsenical, methotrexate, or ionizing or UV radiation) that may have been associated with an increase in carcinogenic risk. The cumulative outpatient use of coal tar medications after initial Goeckerman therapy was recorded.

The characteristics of the patients with and without skin cancers were comparable. Neither group had a history of occupational exposure to known carcinogens. The tendency to sunburn with moderate sun exposure was the same for both groups. Premalignant skin conditions present prior to initiation of the Goeckerman treatment were reported in 10% of patients with skin cancer and 4% of those without skin cancer. More of the patients with skin cancer (67%) reported receiving repeated ionizing radiation treatments than those without skin cancer (58%).

In the Pittelkow et al. (1981) cohort, coal tar usage during the Goeckerman therapy and during subsequent home use was comparable for those individuals who developed skin cancer and those who did

not. The median years of at-home treatment were 21 and 14 for persons with skin tumors and those without skin tumors, respectively (Table 3.1-2). Among those for whom data were available, the percentages of persons using coal tar treatment at home were 56% and 72% in patients with and without skin cancer, respectively, and of those using home treatment, 67% and 56%, respectively, used the product at home for 50 days or more per year (Table 3.1-3).

According to the authors, the mean duration of follow-up was 20.1 years and ranged up to 27 years and the total person-years (the sum of the follow-up times for all patients) was 5,222. Pittelkow et al. (1981) used age-specific incidence rates from the Third National Cancer Survey (TNCS) (Scotto et al. 1974) to calculate the number of persons in whom skin cancer would be expected to develop. An expected number of 26.6 persons was calculated as the average of expected numbers from four geographic areas (49.2 for Dallas-Fort Worth; 15.5 for Iowa; 18.7 for Minneapolis-St. Paul; and 23.1 for San Francisco).

Information was provided on the percentage of coal tar used in hospital therapy and the days of treatment in the hospital setting, and from that information, estimates of grams of coal tar used during a hospital visit could be estimated. Further, the number of patients who continued treatment at home, along with information on the number of years of at-home treatment and the number of treatment days per year, were given such that an estimate of total coal tar exposure could be estimated. The manner in which data were reported in Pittelkow et al. (1981) does not allow that average exposure to be estimated without uncertainty. Consequently, the average exposure was treated as an uncertain parameter and represented, not as a point estimate, but as a distribution of possible values. The distribution of possible average exposure estimates was calculated, as described below, based on uncertain determinants (input parameters) of the exposure scenario, each of which was described by its own distribution of possible values. A Monte Carlo analysis allowed the combination of the uncertain input parameters, yielding the distribution of the average exposure estimate. Details of the calculations are provided here.

For any individual in the cohort, his or her total exposure is given by

$$E_{tot} = E_1 + E_2 + E_h$$

where,

E_{tot} = Total Exposure (grams)
E_1 = Exposure from first hospital treatment (grams)
E_2 = Exposure from second hospital treatment (grams)
E_h = Exposure from at-home treatments (grams)

Each individual's total exposure, E_{tot}, was calculated per the equations and distributions given. Then the average of the 260 E_{tot} values was determined. Based on repeated sampling (300,000 iterations) from the distributions for all of the input parameters, a distribution for the average total exposure, E_{tot}, to members of the Pittelkow et al. (1981) cohort was derived. The mean of that distribution was 254 grams of absorbed coal tar (the median was 252 grams of absorbed coal tar). The distribution of average exposure estimates was used in the dose-response assessment as described in the following paragraphs to derive bounds on the doses associated with specified levels of risk.

In addition to the distribution of average exposures, distributions for the probabilities of response with and without exposure were derived. Those probabilities determine the relative risk for the cohort. The distribution for the probability of response with exposure, *P(d)*, was based on the observed incidence, 19/260; there is a distribution around the most likely value of 19/260 because of the possibility of statistical sampling error. This is completely characterized by the distribution of likelihoods of various values of *P(d)*, making the standard statistical assumption that the observed number of cases arises from an underlying Poisson distribution. A total of 300,000 samples from that distribution was obtained.

The distribution for the probability of response in the absence of exposure, *P(0)*, was centered at the value 26.6/260, which in turn was based on the average expected number of those reported by Pittelkow et al. (1981) as discussed above. The distribution around that value was assumed to be normal for each individual, truncating at the bounds of 15.5 and 49.2, the extremes of the expected numbers reported by Pittelkow et al. (1981). Sampling from the distribution for each of the 260 individuals and summing those contributions yielded a total expected number and, therefore, a probability of response without exposure equal to that expected number divided by 260. This procedure was also completed 300,000 times.

The sets of 300,000 values of total exposure, *P(d)* and *P(0)*, were randomly matched so that a distribution for potency could be estimated. Potency is estimated from the equation

$$RR = 1 + b \times d,$$

where *RR* is relative risk, *b* is the potency factor to be estimated, and *d* is cumulative exposure. Because *RR = P(d)/P(0)*, the above equation can be rearranged to solve for *b*:

$$b = (P(d) / P(0) - 1) / d.$$

The potency distribution obtained by randomly substituting from the 300,000 sets of values for *P(d)*, *P(0)*, and *d* into that equation was used to obtain a conservative, upper bound estimate of the potency, b_u, to use in a lifetable analysis.

Using b_u, and assuming that the relative risk equation above applies at all age levels, the dose corresponding to a lifetime increase in the skin cancer risk equal to 1×10^{-5} was obtained. Note that this dose estimate corresponds to a 95% lower bound on the dose corresponding to that level of risk because of the use of the upper bound estimate for potency. The lifetable analysis yields a background lifetime probability of skin cancer, *LTR(0)*, and a probability associated with a dose rate *D*, *LTR(D)*. Then, the additional risk attributable to exposure to dose rate *D* is

$$Risk = LTR(D) - LTR(0).$$

If that risk is fixed at a level of interest (one in 100,000, i.e., 10^{-5}), and the background rates and potency factor are estimated as described above, the only parameter that is not determined is *D*. Therefore, *D* can be varied until the risk equals the level of interest. In this case, the lifetable analysis applied to these data gives a lifetime background risk of nonmelanoma skin cancer of 0.10. Our upper bound dose-response model (i.e., the lifetable calculation using b_u) predicts that coal tar increases the age-specific incidence rates by a factor of $(1+1.8\times10^{-4}\times d)$, where *d* is the cumulative dose corresponding to the dose rate *D*. When incorporating this information into the lifetable analysis, we assumed that exposure to coal tar occurs at a constant yearly rate throughout life. For *D* equal to 0.0108 grams absorbed/year (or 29 µg absorbed/day), the lifetime risk is 1×10^{-5}. Consequently, the 95% lower bound on the dose predicted to result in one extra cancer in 100,000 persons exposed for a lifetime is 29 µg absorbed/day.

Exposure Assessment

The NSRL estimated using the Pittelkow et al. (1981) data was based on the amount of coal tar ointment absorbed by patients both during their hospital stay and in the years of follow-up home treatment. In comparing exposure to constituents in coal tar from shampooing to this NSRL, the relevant estimate of exposure for the shampoo users is the amount of coal tar absorbed during shampooing. Therefore, the relevant estimate of the LADD to be compared to the NSRL developed using this human data is defined as the amount of shampoo used that is absorbed, accounting for the total amount of shampoo used and the years of shampoo use.

When estimating the LADD, the manner in which shampoo products are used should be considered. Exposure patterns of these products vary among users. The product may or may not be used daily, depending on the nature of the skin condition of the user. Information on product usage is available from consumer surveys, and this can be used to estimate the amount of shampoo used in a year (ounces/year) and duration of use (years). Distributions of product usage can be developed from these data. Moreover, absorbed amounts can be estimated from values published in the literature concerning the amount of a surrogate PAH absorbed during shampooing with a coal tar-containing shampoo. Because of uncertainties in all of these parameters, estimates of exposure for shampoo users have a degree of uncertainty that lend themselves to Monte Carlo analysis. Development of these distributions and use of the available data are described in the following paragraphs.

The LADD in µg/day was calculated using the following equation:

$$LADD_{(\mu g/day)} = \frac{Conc_{mg/g} \times U_{oz/yr} \times CF_{g/oz} \times Y_{yr} \times F \times 1000\ \mu g/mg}{70\ yr \times 365_{days/yr}}$$

where,

LADD	=	Lifetime Average Daily Dose (in µg/day)
$Conc_{mg/g}$	=	mg of coal tar/gram shampoo
$U_{oz/yr}$	=	ounces of shampoo used/year
$CF_{g/oz}$	=	grams/ounce conversion (28.35)
Y_{yr}	=	number of years of usage
F	=	fraction of coal tar in applied shampoo that is absorbed
1000	=	µg/mg conversion
70	=	years in a lifetime
365	=	days/year

Distributions were developed for the parameter ounces of shampoo used per year (*U*), the number of years of usage (Y) and the fraction of coal tar in the applied shampoo that is absorbed (*F*) for use in a Monte Carlo analysis to provide a distribution of LADDs.

A Monte Carlo analysis based on the equation to estimate the LADD was conducted using those distributions, and the data output for that analysis are found in Appendix E. Under Proposition 65 in the State of California, determination of the need to warn is based on exposure to the typical product users. Therefore, the median values from the distribution of LADDs were selected and are reported in Table ES-1 for coal tar-containing shampoos with a percent coal tar ranging from 0.5% up to 2.5%. The estimated median LADDs ranged from approximately 1 µg absorbed/day at 0.5% to 5 µg absorbed/day

for the 2.5% product. These values are compared to the NSRL derived from the data reported by Pittelkow et al. (1981).

Table ES-1
Estimates of Lifetime Average Daily Dose (µg/day) from Exposure to Coal Tar in Shampoo from Monte Carlo Analysis of Exposure through Shampoo Use

% Coal Tar in Shampoo	Median (50th percentile) (µg/day)
2.5%	5.1
2.0%	4.1
1.8%	3.7
1.6%	3.3
1.4%	2.9
1.2%	2.5
1.0%	1.7
0.5%	1.0

Risk Characterization

A dose-response analysis was based on epidemiological data for a group of patients treated with coal tar for psoriasis at the Mayo Clinic (Pittelkow et al. 1981). Using exposure information published about the cohort as well as information in general about the treatment modalities at the Mayo Clinic and other psoriasis clinics using coal tar ointment therapeutically at that time, estimates of the amount of total coal tar used by each patient over their course of treatment were estimated. Using a Monte Carlo analysis, with uncertainty distributions for the parameters determining total exposure and the probabilities of response with and without exposure, an upper bound potency estimate was derived. A lifetable analysis conducted with the upper bound potency provided an estimate of the 95% lower bound on the lifetime dose associated with the target risk level, which for the purposes of this analysis, was one extra cancer in 100,000. That dose was 29 µg absorbed/day.

Estimates of the LADD, again using Monte Carol analyses, provided estimates of exposure to coal tar-containing shampoo users for coal tar content ranging from 0.5% to 2.5%. These values ranged from approximately 1 µg absorbed/day for the 0.5% coal tar shampoo up to 5 µg absorbed/day, all of which are lower than the target dose of 29 µg absorbed/day. The ratio of the estimated exposure from long-term use of coal tar-containing shampoos to the NSRL, which is the 95% lower bound on the lifetime dose at the 1×10^{-5} level, is less than one.

Consideration of Uncertainties

Uncertainties in the use of the Pittelkow et al. (1981) study and the use of the mouse bioassays to develop NSRLs were considered. Uncertainties regarding the estimates of exposure to coal tars from the use of coal tar-containing shampoos were also considered and discussed in detail in Section 5.2. Only a brief summary of key uncertainties is provided here.

Uncertainties Related to the Dose-Response Model Using Human Data. The first "uncertainty" is that associated with the use of the Pittelkow et al. (1981) cohort as the basis for the risk assessment. The relevant uncertainties in this regard concern the appropriateness of the cohort as a sample from the general population. If it can be considered to provide a fair representation of what would be generally expected under the specific coal tar exposure conditions under study, then it must be considered an appropriate basis for the risk assessment. The data suggest that use of this population is relevant and predictive for persons using coal tar-containing shampoos. As discussed, psoriatic and normal skin compare favorably with regard to absorption, metabolic capability, DNA adduct formation and repair, and a lack of ODC induction following coal tar application. The general consensus from studies in which the incidence of skin cancer was compared to the incidence rate in the general population was that psoriasis did not confer either protection or increased sensitivity (Alderson and Clark 1983; Bhate et al. 1993; Stern et al. 1985). An increased incidence of skin cancer in psoriasis patients was associated with other forms of treatment, in particular PUVA. A second reason is that persons using coal tar-containing shampoos are also using these products for medicinal reasons to treat psoriasis or other skin conditions of the scalp. Therefore, it is expected that use of this population to derive a NSRL for coal tar in anti-dandruff shampoos will be protective of coal tar-containing shampoo users.

The second uncertainty associated with the use of the Pittelkow et al. (1981) study concerns the possibility that not all of the skin cancers that the cohort developed have been reported in that study. This concern in relation to the reported count of skin cancers is treated quantitatively (and discussed below). In relation to the time since follow-up ended, note that both the observed number and the expected number of skin cancers would be affected, so there is no known bias introduced by considering only the follow-up period presented in Pittelkow et al. (1981). The follow-up time for the cohort as reported in the 1981 report had reached as much as 27 years, so this may not be a source of great concern.

Each of the uncertain parameters contributing to the calculation of a patient's total exposure to coal tar was assigned a particular distribution to describe its uncertainty. This is the essence of the Monte Carlo procedure. Nevertheless, the exact nature of that distribution (e.g., whether a triangular distribution

is a better descriptor of the uncertainty than a truncated normal distribution) is not known. Therefore, the assumptions made concerning those distributions must be recognized as uncertainties of the analysis. Through a formal sensitivity analysis, the parameters that were most uncertain and to which the potency estimates were inherently most sensitive were considered further in an uncertainty analysis. It was determined that, when reasonable alternative choices for the uncertainty distributions for those parameters were selected, the quantitative effects are such that the resulting alternative NSRL estimate is never less than or equal to the exposures determined for the typical shampoo users. Some of the alternative NSRLs increased to infinity, implying that no exposure level is estimated to yield a risk as high as one in 100,000.

A linear relationship between total exposure and relative risk has been assumed. That is not the only relationship that could have been used. However, with a single treated group (Pittelkow et al. (1981), the options for estimating alternative relationships are limited: only a single unknown parameter can be estimated (the potency factor, *b*, was the unknown parameter estimated from the Pittelkow et al. data). The choice of a linear relationship is recognized as a "conservative" choice in cancer risk assessment practice. Therefore, while the use of the linear relationship for relative risk is an uncertainty, it is not likely to contribute to an underestimation of low-dose risk.

Once the relationship between relative risk and total exposure had been determined for the Pittelkow et al. (1981) cohort, that relationship had to be "extrapolated" to the general population via the lifetable calculations. To do so, it was assumed that the estimated relationship held for all ages. That is, the relative risk was assumed to be constant, for any given value of total exposure. This is a standard assumption used in most risk assessments.

As stated, all of the above-mentioned uncertainties are present in the analysis performed here. They are typical of the uncertainties inherent to most, if not all, risk assessments. They are not necessarily quantifiable in the sense of being able to yield confidence limit statements; the quantifiable uncertainties associated with estimation of input parameters have been considered in the derivation of the conservative bounds on risk or dose and the effects of alternative uncertainty distributions have been examined.

Uncertainties Related to Estimates of Exposure. Estimation of the LADD for shampoo users required four pieces of information: concentration of the coal tar in the shampoo, ounces per year of shampoo used, years of shampoo usage, and fraction of coal tar in shampoo that is absorbed. The concentration of coal tar in the shampoo was assumed to cover a range of concentrations from 0.5% to

2.5% of the shampoo. These are based on actual concentrations found in shampoo. Estimation of a LADD for a product with a concentration outside of this range is a simple linear extrapolation, given the assumptions that have been used.

The ounces per year of coal tar use and the years of usage were derived from survey data (Nielsen 1999; Toppmeyer 1994) with support from published literature sources (Menter and Cram 1983). While the population size used to estimate the ounces of shampoo used per year (Nielsen 1999) was relatively large (nearly 3200 households), the data for the number of years of usage were from smaller populations and required a regression approach to derive values appropriate for a 70-year lifespan. Nevertheless, both years of use and ounces of shampoo used per year have most likely been overestimated (and therefore the exposure to the typical user overestimated) because of the surrogates used to derive the distributions. In the case of years of use, the surrogate was years of suffering from a scalp condition requiring a dandruff shampoo; for some of that time individuals would not have treated the condition or would have used dandruff shampoos without coal tar. In the case of ounces of shampoo used per year, the surrogate used was household purchases, which overestimates use if any of the purchased material goes unused or if more than one person per household uses the shampoo.

A major uncertainty in the estimates of exposure to persons using coal tar-containing shampoos was that absorbed dose was calculated from data for 1-OH-P in the urine of volunteers who used a coal tar-containing shampoo (van Schooten et al. 1994). This is uncertain for two main reasons. The first is the use of 1-OH-P, the major metabolite of pyrene, as a biomarker of exposure to PAHs. The data indicate that use of pyrene as a surrogate for absorption of the PAHs of interest, namely the higher molecular weight PAHs that are considered to be the carcinogenic constituents, would result in an overestimate of absorption for BaP by at least a factor of 3, based on a 24-hour exposure, and a factor of 7 for about a 4-hour exposure. Other PAHs would be overestimated by a much greater amount (Dankovic et al. 1989; Roy et al. 1987; Van Rooij et al. 1995). Consequently, the amount of absorption may be overestimated. 1-OH-P data were relied upon for estimating the absorbed dose in both the dose-response and exposure assessment. Therefore, if absorption is overestimated for the dose-response assessment, it is likely overestimated similarly for the exposure. It is expected that although the use of pyrene data to estimate absorption of constituents from coal tar may be an overestimate for constituents other than pyrene, the relative absorption between pyrene and other coal tar constituents following ointment or shampoo usage will be similar.

The second uncertainty in using urinary 1-OH-P levels to estimate the absorption fraction is that data on multiple steps are needed that include the amount of pyrene applied to the skin and how much of

the amount of pyrene absorbed is metabolized to 1-OH-P and excreted in the urine. No one study quantified each of these components, and a wide range of values was derived based on the literature. Nevertheless, use of this wide range in a Monte Carlo analysis should not result in an underestimate of exposure to the shampoo users.

Summary of Uncertainties. The potential impact of the uncertainties listed here should be considered in the risk characterization and may legitimately influence the decision-making process. Note, however, that many of the uncertainties confronting most risk assessments, primary among them being those associated with extrapolation from animal test species to humans, are absent from this risk assessment. Moreover, it does not appear that any of the uncertainties discussed here would have such an impact as to negate the results of this analysis. That is, since some purposely conservative choices have been made in this analysis, the resulting estimates are not likely to underestimate the risk of skin cancer associated with exposure to coal tar.

Conclusions

As in any risk assessment, there are uncertainties associated with the estimates of risk discussed above. One strength of the analysis as presented here is that uncertainties associated with quantitative estimation [e.g., of the average cumulative exposure for the members of the Pittelkow et al. (1981) cohort, or of the underlying probability of nonmelanoma skin cancer in that cohort] have been incorporated into the analysis methodology. As a result, the analysis results have been able to provide an estimate of the risk of using coal tar-containing shampoo that appropriately reflects the level of confidence that can be associated with the input parameters. In fact, the analysis has provided an estimate of the risk that is conservative in a definable sense: given the data now available and the quantitative uncertainties associated with estimation of the underlying realities (e.g., estimation of the true probability of response), the conservative estimate of risk is one that underestimates the true risk with a defined and small probability (i.e., with probability less than 0.05). Conversely, the conservative estimate of dose associated with a target risk is one that overestimates the true dose associated with that risk with a defined and small probability (0.05).

The purpose of this project was, in the context of a quantitative risk assessment, to compare estimates of the LADD of coal tar constituents that can be expected following use of coal tar-containing shampoos according to the user scenarios defined by the distributions of the concentration of coal tar in

shampoos, the amount of coal tar-containing shampoo used (on a yearly basis), the number of years of usage, and the amount of absorption. The NSRLs estimated based on either the human epidemiology data or the mouse bioassay data were larger than the corresponding LADD for all concentrations of coal tar-containing shampoos considered. Estimates of exposure to persons regularly using coal tar-containing shampoos were below the 95% lower bound on dose at the target risk level of one in 100,000.

1.0 Introduction

Pharmaceutical grade coal tar-containing ointments have been used in the treatment of psoriasis, seborrhea, and other skin conditions for almost 100 years. More recently, pharmaceutical grade coal tar-containing shampoos have been used to treat psoriasis, seborrhea and/or protracted dandruff of the scalp and numerous coal tar-containing shampoos have been approved by the Food and Drug Administration (FDA) for medicinal use. However, constituents in pharmaceutical grade coal tar, in particular benzo(a)pyrene (BaP) and other large polycyclic aromatic hydrocarbons (PAHs), are known to initiate and/or promote skin tumors in laboratory animals following dermal application. Results from some occupational studies indicate that there is an increase in the risk of skin cancer in workers following exposure to high concentrations of PAHs, especially in workers exposed to sunlight or chronic irritation and/or poor hygiene practices. However, no clear increased incidence of skin cancer has been reported in psoriasis patients who have been exposed to therapeutically high doses of pharmaceutical grade coal tar (Dodd 1993; Pion et al. 1995; van Schooten and Godschalk 1996). According to van Schooten and Godschalk (1996), "skin tumors induced by therapeutic use of coal tar are seldom seen in the clinical procession." According to Dodd (1993), "despite the theoretical potential of tars to be carcinogenic, from the dermatologists' clinical experience, the risk of skin cancer produced by tar preparations as used in psoriasis therapy appears to be very small."

As a consequence, there are apparently two diametrically opposing views with regard to coal tar in medicinal ointments. On one side is the view based on the animal data that dermal exposure to constituents in coal tar may pose an unacceptable risk of skin cancer in persons using medicinal products containing coal tar. On the other side is the view that coal tar ointments have been used extensively and safely for more than 100 years. Further, it is argued that if the use of large quantities of coal tar to treat psoriasis patients does not pose an unacceptable risk of skin cancer, then the use of coal tar-containing shampoos for which both the quantity of material used and the duration of time the material is in contact with the skin are significantly smaller, then use of coal tar-containing shampoos should also be safe. Both of these positions are qualitative positions. To date no quantitative skin cancer risk assessment that includes an evaluation of the toxicity of pharmaceutical grade coal tar ointment, the extent of exposure from coal tar-

containing shampoos, and the lifetime extra cancer risk of skin cancer from the use of coal tar-containing shampoos has been conducted.

The purpose of this investigation was to conduct a quantitative risk assessment to assess the potential for skin cancer in persons who regularly use pharmaceutical grade coal tar-containing shampoos. The technical approach that was used followed, when the data allowed, the conceptual approach proposed in the USEPA's Human Cancer Risk Assessment guidelines (USEPA 1996, 1999). Moreover, because the context of this risk assessment is the question of a warning label under California's Proposition 65 statutes, the target population was defined as the average coal tar-containing shampoo user and the target risk was one in 100,000.

When based on animal data, the carcinogenic potency of a chemical is based on the type of tumor in the animal model that maximizes the biological comparability with humans. When that is not known, the potency is based on the endpoint that produces the highest estimate of potency. However, when other evidence is available, then evaluation of the potential for carcinogenicity of a compound requires an integration of the statistical and toxicological significance of the observations in these bioassays to determine: 1) the relevance of these observations to human health, and 2) for those responses that are relevant to humans, the dose-response for that endpoint. The evidence for coal tar falls into this category. Consequently, the Technical Hazard Assessment consisted of a weight-of-evidence characterization of the carcinogenic potency of pharmaceutical grade coal tar and considered two general types of evidence: 1) primary evidence, which included epidemiological studies and lifetime animal bioassays (Sections 2.1 and 2.2); and 2) secondary evidence, which included consideration of data generated to evaluate pharmacokinetics and mechanism/mode of action that may direct selection of the data to be used in the dose-response analyses (Sections 2.3, 2.4, 2.5).

The Dose-Response Assessment (Section 3.0) initially considered using data from clinical epidemiological studies that evaluated the incidence of skin cancer in patients treated with coal tar-containing ointments for psoriasis and using animal bioassay data. With the use of the clinical epidemiological data, estimates of the coal tar exposure for these patients both during their hospital stay and during the years of at-home use of coal tar would be required and could be based on several clinical sources. If the use of the mouse bioassay data in dose-response modeling was considered relevant, then the selection of the relevant dose-metric that would be

assumed to engender equivalent risks across species when the route of exposure was the dermal route and skin was the target tissue would be required. Although USEPA initially proposed an approach (USEPA 1997), currently, no one candidate (i.e., mg/cm^2, mg/kg/day) has been selected as the relevant dose-metric for species extrapolation when evaluating the risk of skin cancer from the dermal route of exposure (USEPA 2000). When assessing selection of a dose-metric to use with the animal data, species differences that would impact cross-species extrapolation (e.g., pharmacokinetic) need to be considered, as well as the manner in which these data could be used quantitatively to derive a lower bound on dose at the target risk.

The Exposure Assessment (Section 4.0) for persons who use pharmaceutical grade coal tar-containing shampoos was characterized using product-specific and user-specific data. Both consumer surveys and data in the published literature were used to derive estimates of the LADD to which the lower bound on dose at the target risk could be compared (Section 5.0). Estimates of exposure both to the psoriasis patients, which was used in the dose-response assessment, and to coal tar-containing shampoo users, which was used to assess the risk to these persons, were complex. Multiple sources of data were available, some of which were in close agreement, such as the number of years of coal tar treatment for psoriasis, while other data were quite variable, such as the number of days of at-home treatment. Consequently, Monte Carlo analyses were conducted to provide a distribution of estimates of exposure for both the psoriasis patients and shampoo users.

This was an investigation that required a re-examination of the underlying assumptions regarding the toxicity of coal tar materials and constituents in coal tar, as well as a re-examination of how those data could be used in a quantitative risk assessment. A Risk Characterization (Section 5.0) based on this investigation includes a discussion of the major uncertainties in this assessment and the impact of these uncertainties on the interpretation of the potential lifetime extra skin cancer risk to persons who use coal tar-containing shampoos.

2.0 Technical Hazard Characterization

The term “coal tar” is used to describe a variety of complex mixtures, including pharmaceutical grade coal tar, creosote and coal tar pitch, that are formed by the distillation of coal (IARC 1985). These complex mixtures consist of PAHs, other hydrocarbons, phenols, and heterocyclic nitrogen, sulfur and oxygen compounds. In the distillation process, because of the different physicochemical properties of the individual constituents, each constituent in coal will distill or fractionate within a narrow temperature range. Consequently, the chemical composition of a specific coal tar mixture, e.g., both the types and amounts of the constituents present, is dependent on the distillation temperature at which the coal tar fraction was recovered and the composition of the feed stock. Therefore, a coal tar fraction captured at a low temperature range, such as creosote, which is representative of the fraction eluded at 300 to 400 degrees, will have a different chemical profile than a coal tar fraction eluded at a higher temperature, such as pharmaceutical grade coal tar, which is the representative fraction distilled in the 900 to 1100 degree range. Conversely, coal tars collected in a specific temperature range will have very similar chemical profiles (IARC 1985; Wright et al. 1985).

Given the differences in chemical composition between pharmaceutical grade coal tar, creosote and coal tar pitch, it would be expected that the potential toxicity of these mixtures would vary. Mixtures formed at lower distillation temperatures would be expected to have higher concentrations of lower molecular weight PAHs, many of which are irritants, while mixtures created at higher temperatures would have more of the larger molecular weight constituents, some of which have been classified as carcinogens. However, the potential toxicity of any given mixture is a function of the complex interactions of the constituents in the mixture, in that individual constituents may enhance or inhibit the action of another constituent such that the toxicity of the mixture can not be defined by one constituent or the summation of a subgroup of constituents (Goldstein et al. 1998; Warshawsky et al. 1993; Wright et al. 1985). Consequently, evaluation of toxicity should consider the specific coal tar mixture tested rather than the individual constituents in that mixture. Further, it is expected that while mixtures created at different distillation temperatures would have different toxicity profiles, it is also expected that mixtures created at the same temperature range would not only have similar

chemical profiles, but similar toxicological properties. Consequently, while some variation across batches of pharmaceutical grade coal tar may exist, it is expected that both the chemical composition and toxicological potential of pharmaceutical grade coal tars in various consumer products are more similar than that for different coal tar mixtures, such as creosote. Therefore, in this Technical Hazard Characterization, the mixture tested was a consideration in the evaluation of the suitability of data for use in estimating the risk associated with the use of pharmaceutical grade coal tar-containing shampoos.

2.1 Animal Studies: Dermal Route of Exposure

Clear evidence of carcinogenicity for individual constituents in coal tar has been provided in numerous initiation/promotion skin painting studies in mice (ATSDR 1996; Nesnow et al. 1982; Warshawsky et al. 1993; Wright et al. 1985). Other skin painting studies have evaluated the carcinogenic potential of coal tar mixtures including coal tar distillates, as well as coal tar fractions isolated from emissions produced by coke ovens, roofing tar, and coal-fired residential furnaces (Boutwell and Bosch 1958; Cabot et al. 1940; Fraunhofer 1997; Grimmer et al. 1984; Lijinsky et al. 1957; Nesnow et al. 1982; NIOSH 1979; Warshawsky et al. 1993; Wright et al. 1985). Although each of the products tested contained coal tar or coal tar constituents, the composition of the mixtures applied varied considerably with each material tested. The review of the animal studies focused on lifetime bioassays in which a coal tar-containing material was dermally applied for the duration of the study. The intent of this review was to: 1) identify studies for dose-response modeling, and 2) consider the differences in the content of the mixtures tested and how the variations in content potentially affected activity.

2.1.1 Skin Painting Studies Evaluating Pharmaceutical Grade Coal Tar

Few studies were identified in which either USP pharmaceutical grade coal tar (Mukhtar et al. 1986; Wright et al. 1985) or therapeutic coal tar-containing ointment (Shabad et al. 1970) was tested and of these, none provided dose-response data. In the study conducted by Shabad et al. (1970), only one dose group was tested and no control group was included. After a year of application of a coal tar-containing ointment (100 mg ointment/application for 186 applications), 14/18 animals developed one or more skin tumors at the application site. Both of the studies by

Mukhtar et al. (1986) and Wright et al. (1985) were initiation/promotion studies in which a single application of one dose of pharmaceutical grade coal tar was applied to the shaved skin of mice followed by a weekly applications of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). In Mukhtar et al. (1986), application of coal tar in SENCAR mice was compared to application of BaP and 7,12-dimethylbenz(a)anthracene (DMBA). The authors concluded that the coal tar material was a relatively weak tumor initiator compared to the individual PAHs in that both the latency time (time-to-tumor) was longer and the number of tumors per mouse was less; however, the amount of individual PAH constituents in the coal tar tested was not given.

Wright et al. (1985) conducted an initiation/promotion study in which the coal tar portion from a 20% pharmaceutical stock material and an industrial coal tar supplied by the National Bureau of Standards (NBS) were evaluated in a mouse skin painting assay. A single application of 25 mg of either the NBS or pharmaceutical stock coal tars was applied twice weekly to the shaved backs of CD-1 mice in a methylene chloride vehicle followed by the promoter, phorbol myristate acetate, for the duration of the study. No control group was included in this study. At 57 days post-initiation, tumor incidence was approximately 15% and 30% in the pharmaceutical stock and NBS groups, respectively. A tumor incidence of 100% was reported at 169 days post-initiation for crude NBS coal tar and coal tar from pharmaceutical stock material. However, the cumulative number of tumors normalized for 30 mice/group was greater in the NBS coal tar group than in the pharmaceutical stock group, approximately 180 and 140, respectively, at 169 days. The total PAH content in the NBS coal tar and the pharmaceutical stock material was 643.18, and 565.03 parts per thousand, respectively, approximately a 12% difference in PAH content between test materials. However, the BaP content in the pharmaceutical stock solution was approximately 69% less than the BaP content in the NBS coal tar. Although each coal tar mixture contained different BaP fractions, similar tumor incidences were observed. Therefore, the results of this study suggested that the BaP content alone may not have been responsible for the activity, but that other constituents in the coal tar products (CTPs) played a role in carcinogenesis in mouse skin.

2.1.2 Skin Painting Studies Evaluating Different Coal Tar-Derived Mixtures

Mouse skin painting studies for other coal tar-derived mixtures were reviewed. While other studies included in this evaluation provided qualitative support (Grimmer et al. 1984; Mukhtar et al. 1986; Nesnow et al. 1982; NIOSH 1979; Warshawsky et al. 1993; Wright et al. 1985), data were not available to perform quantitative analyses. Of the studies reviewed, only the study conducted by the Fraunhofer Institute of Toxicology (1997) provided dose-response information. Therefore, these studies were used to provide information on the differences in content of coal tar mixtures and how the variations in content affected tumor activity.

2.1.2.1 Coal Tar Distillate: Creosote

The Fraunhofer Institute of Toxicology (1997) conducted a mouse skin painting study where the potential dermal carcinogenicity of two different coal tar mixtures (100% coal tar distillate), designated as CTP1 (low BaP content) and CTP2 (high BaP content), was evaluated. In this study, 0 (toluene vehicle control), 0.1 (CTP2 only), 0.3, 1, 3 or 9 mg (25 Fl toluene/treatment) of CTP1 or CTP2 was applied to the shaved interscapular skin of groups of 62 male CD-1 mice [Crl:CD®-1(ICR)BR strain], approximately 6 weeks of age at initiation of treatment, twice a week for 78 weeks. An additional group of 62 mice received 7.5 Fg toluene/day of the positive control, BaP. The study authors noted that some of the mice developed ulceration at the application sites. In these animals, treatment was discontinued, the ulcerated areas allowed to heal and treatment was then resumed. However, all animals in the high-dose (9 mg) CTP2 group were sacrificed during weeks 38 and 39 of the study due to persistent and suppurative ulceration. After treatment, all remaining surviving animals in all groups were sacrificed, and treated areas of the skin were collected and examined microscopically. In order to consider the potential impact of survival on tumor development, a lifetable analysis was conducted.

The coal tar solutions (CTP1 and CTP2) contained the PAHs in the concentrations listed in Table 2.1-1. The primary differences between the two coal tar solutions were BaP content (10.4 Fg/g in CTP1 compared to 281 Fg/g in CTP2), total PAH content and BaP-equivalents.[1] The total PAH concentration and BaP-equivalents was 41,037 and 16.6 Fg/g, respectively, in

[1]Derived using CalEPA's potency equivalent factor (PEF) (CalEPA 1994).

CTP1, compared to 226,451.4 and 478 Fg/g, respectively, in CTP2. Thus, BaP content, total PAH content and BaP-equivalents were higher by approximately a factor of 27, 5.5, and 29, respectively, in CTP2, when compared with CTP1.

Results - CTP1. Survival was significantly decreased in the high-dose (9 mg) CTP1 group, with mean survival times of 70 weeks and 64 weeks in the vehicle control and 9 mg groups, respectively, when compared to the other treatment groups. However, the results of the lifetable analysis indicated that the shorter lifespan in the high-dose CTP1 group did not significantly influence tumor development. The skin tumor incidence data are presented in Table 2.1-2. There were no statistically significant increases in the incidence of skin tumors in any group treated, when compared with the vehicle control. Statistically significant increases in the incidences of squamous cell carcinoma (SCC) or papilloma were reported in the positive control group.

Increases in the incidence of several nonneoplastic lesions of the skin were also reported in the animals treated with CTP1. The incidence of selected lesions and the severity scores of each lesion are summarized in Table 2.1-3. Lesions evaluated included the incidence of epidermal hyperplasia with cellular atypia, epidermal hyperplasia, purulent dermatitis (superficial), hyperkeratosis, and ulcerative dermatitis (ulceration). A statistically significant increase in the incidence of severe ulcerative dermatitis was observed in mice that received 1 mg CTP1 or greater, when compared to negative controls.

Results - CTP2. Survival was significantly decreased in mice that received 1, 3 or 9 mg CTP2, with mean survival times of 63, 58 or 36 weeks (sacrificed due to persistent ulceration), respectively, compared to 70 weeks for the vehicle controls. Statistically significant increases in skin tumor incidence were reported in mice that received CTP2 (Table 2.1-4). Specifically, the incidence of SCC was significantly increased in mice that received 3 or 9 mg CTP2, when compared with the controls, while the incidence of papilloma was significantly increased in mice that received 1 mg CTP2 or greater. The incidences of SCC and papilloma were also increased in the positive control group. The incidence of selected lesions and the severity scores of each lesion are summarized in Table 2.1-5. A statistically significant increase in the incidence of severe epidermal hyperplasia with cellular atypia and severe epidermal hyperplasia was observed in the 3 and 9 mg treated groups, when compared to controls. The incidence of severe

hyperkeratosis and severe ulcerative dermatitis was also statistically significantly increased in the 1, 3 and 9 mg groups, when compared to controls.

2.1.2.2 High Temperature Coke Oven Coal Tar

In a set of three experiments, Warshawsky et al. (1993) investigated various combinations of individual PAHs, including anthracene, chrysene, fluoranthene, phenanthracene, pyrene, with and without BaP, or a high temperature coke oven coal tar containing BaP (Tables 2.1-6 and 2.1-7). In all experiments, PAHs or coke oven coal tar were applied in a toluene or n-dodecane base twice weekly to the shaved skin of CH_3 mice (20/group) for 2 years. In one experiment, groups of 20 mice received 50 Fl of solutions that contained: 1) 0.0006% BaP diluted in toluene, 2) coal tar that contained 0.0006% BaP diluted in toluene, 3) 0.0006% BaP in toluene followed by 50 Fl n-dodecane, or 4) coal tar that contained 0.0006% BaP diluted in toluene followed by 50 Fl n-dodecane, a tumor promoter. As shown in Table 2.1-6, neither the application of 0.0006% BaP in toluene nor 0.0006% BaP in toluene plus 50 Fl n-dodecane produced tumors; however, 51% of the mice had tumors in the group that received coal tar containing 0.0006% BaP in toluene. The addition of n-dodecane increased the tumor incidence to 65% and decreased the latency period from 73 to 39 weeks. Thus, the activity of the mixture could not be attributed to the BaP content alone.

In a second experiment, groups of 20 mice received 50 µl volumes of: 1) toluene solutions that contained 0.1% of each of anthracene, chrysene, fluoranthrene, phenanthrene or pyrene, 2) 0.1% toluene solutions that contained each of the five PAHs plus 0.001% BaP, 3) a toluene solution that contained 0.1% of each of all five PAHs, and 4) a toluene solution that contained 0.1% of all five of the PAHs plus 0.001% BaP. The addition of BaP to the individual PAH solutions resulted in an increased tumor incidence and a decreased latency period, although the application of 0.001% BaP alone did not result in tumor formation (Table 2.1-6). A tumor incidence of 7% and a latency period of 81 weeks were reported in mice that received 0.1% chrysene alone. However, in mice that received 0.1% chrysene plus 0.001% BaP, the tumor incidence was 23%, while the latency period was 70 weeks. Similar findings were reported for anthracene, fluoranthrene and phenanthrene. One skin tumor was observed following the application of a 0.1% pyrene solution; however, no tumors were observed following the

application of 0.1% pyrene plus 0.001% BaP. Thus, with anthracene, chrysene, fluoranthrene and phenanthrene, but not pyrene, the addition of BaP influenced the activity and the latency period despite the lack of a response following the application of BaP alone.

In a third set of experiments, mice (20/group) were dermally exposed twice weekly for 2 years with 50 µl of 0.2% of a series of benz(a)anthracene (BA) derivatives, including 9-methyl-, 2-methyl-, 3-methyl-, 8-methylbenz[a]anthracene, and dibenzanthracene, in toluene, dodecane, or 30% toluene and 70% n-dodecane. Additional groups of animals were left untreated, treated with toluene only or treated with n-dodecane only and served as controls.

There were no tumors reported in mice that received toluene or n-dodecane vehicles or the 0.2% solutions of 9-, 2-, or 3-methylbenz[a]anthracene in toluene (Table 2.1-7). However, when these chemicals were applied in an n-dodecane vehicle, incidences of 76%, 44%, and 88%, respectively, were reported. The 8-methylbenz[a]anthracene and dibenzanthracene were carcinogenic in toluene, with incidences of 94-95% and latency periods of 41 and 35 weeks, respectively, reported. However, when applied in n-dodecane, the tumor incidence was 100%, with latency periods of 25 and 26 weeks reported for 8-methylbenz[a]anthracene and dibenzanthracene, respectively. Thus, n-dodecane enhanced the activity and the latent period of the PAHs tested.

The Warshawsky et al. (1993) study was designed to illustrate factors affecting the carcinogenic potential of complex PAH mixtures. Following a review of the results, the authors concluded that the "presence of BaP cannot account for the observed potency of mixtures." They further concluded that the specific "interaction of individual PAHs with BaP is dependent on the PAHs studied." Lastly, the authors concluded that "the activity of the coal tar must be due to additional carcinogens or cofactors in solution that are present in coal tar."

2.1.2.3 Roofing Tar and Coke Oven Particulates

Nesnow et al. (1982) conducted an initiation/promotion study in SENCAR mice evaluating the tumor initiating activity of roofing tar and coke oven particulate emissions and BaP. Greater than 99% of organic solubles from roofing tar (889 ng/mg extract) were extractable; however, only 7% from the coke oven (478 ng/mg extract) sample was extractable. Roofing tar and coke oven samples were applied topically to 7- to 9-week-old SENCAR mice

(40/sex/group) at quantities of 0.10, 0.50, 1.0, 2.0, or 10.0 mg in 0.2 ml acetone. BaP was applied at quantities of 2.5, 12.6, 50.5 or 100.1 µg in acetone. One group of mice was topically treated with 0.2 ml of acetone only and served as the vehicle controls. One week following treatment, all mice were topically administered 2.0 µg of TPA in 0.2 ml acetone, twice weekly for an unspecified number of weeks.

Results indicated a strong dose-related tumor response in coke oven and roofing tar emissions. A dose-related tumor response was also observed in mice treated with BaP. At the highest quantity tested (10 mg), papillomas were observed in 100% of male and 95% of female mice in the roofing tar treatment group and 100% of male and female mice in the coke oven emission group. A higher incidence of papillomas was observed in the coke oven groups treated with 2.0 (95% males, 90% females), 1.0 (95% males, 72% females), and 0.5 mg (73% males, 70% females) of sample, when compared to roofing tar groups treated with 2.0 (36% males, 37% females), 1.0 (38% males, 45% females), or 0.50 mg (28% males, 13% females) of sample. Similar incidences of papillomas were observed in animals in the 0.10 mg roofing tar and coke oven treatment groups. However, the BaP content was consistently higher in roofing tar than in coke oven tar. For example, the BaP content in the 0.1 mg roofing tar sample was approximately 4.8 µg BaP, compared to 4.8 ng BaP in the coke oven tar. The incidences of papillomas in the 2.5, 12.6, 50.5, and 100.1 µg BaP/mouse treatment groups were 45%, 73%, 100%, and 95% for males, respectively, and 31%, 57%, 75%, or 97% for females, respectively. Therefore, when comparing the incidence of papillomas in the BaP treatment groups versus the incidence of papillomas in the roofing tar and coke oven treatment groups, the incidence of tumors varied, and was not related to the BaP content alone. For example, BaP applied at a quantity of 2.5 µg/mouse resulted in a 45% or 31% incidence of papillomas in male and female mice, respectively. However, the 2 mg/mouse coke oven sample, with similar BaP content (4.8 µg BaP), resulted in a much larger incidence of papillomas for male (95%) and female (90%) mice. Also, similar BaP content in both roofing tar and coke oven samples resulted in very different tumor incidences. The 2 mg of roofing tar or coke oven sample had similar BaP content (1.8 µg or 0.9 µg, respectively), but produced distinctly different incidences of papillomas (36% males, 37% females or 95% males, 90% females, respectively). The results of this study suggest that

even with similar BaP content in the test material, differing tumor incidence is observed depending on other constituents in the mixture.

2.1.2.4 Coal and Coal Tar Subfractions

Data have also been reported that suggest that PAHs with three or more aromatic rings serve as the major contributors to the observed carcinogenicity from coal tar. Grimmer et al. (1984) conducted a study to identify components of and determine the potential carcinogenicity of emission condensates from coal-fired residential furnaces using CFLP mice. Various fractions of the emissions were evaluated for carcinogenicity by comparing the fractions to the proportion to total condensate (wt%). The results of Probit and Weibull analyses indicated that the PAHs and thiaarenes with more than three aromatic rings accounted for virtually all of the total carcinogenicity of the condensate. BaP contributed 10-11% of the total carcinogenicity of the emission condensate even though BaP was only a small fraction (0.702 mg/g condensate) of the total condensate. However, the polyaromatic compounds (PAC)-free fraction, and the fraction containing PACs with two and three rings were essentially without activity.

NIOSH (1979) conducted a study to determine the biologic activity of 16 coal tar subfractions of a composite coal tar material through the use of a mouse-skin bioassay utilizing C3H/HeJ male mice. However, due to the expiration of the project, only seven subfractions were able to be tested. The seven subfractions included Subfraction 2: approximately 50% naphthalene; Subfraction 3: probably a mixture of naphthalene, substituted naphthalenes, and anthracene; Subfraction 6: possibly substituted three-ring systems with some four-ring compounds; Subfraction 7: possibly pyrene, phenanthrene, and substituted three-ring compounds; Subfraction 9: mostly condensed four-ring systems, with possibly BA and/or chrysene as major components; Subfraction 10: probably five-ring systems and higher, possibly BaP; and Subfraction 13: possibly polar polynuclear compounds. Squamous cell papillomas were observed in two, six and one animal from Subfraction groups 7, 9, and 13, respectively. SCCs were reported in five and six animals from Subfraction groups 9 or 10, respectively. There were no tumors reported for animals in the control group or animals in any other subfraction group. Overall, this study showed that the four- and five-ring PAHs produced the greatest biologic activity, with no carcinogenic tumors produced by PAHs with three rings or less.

2.1.3 Comparison of Mouse Strains

The development of skin tumors in mice consists of the initiation (DNA damage such as the formation of DNA adducts) of epidermal cells and the promotion of these initiated cells. Some strains of mice are more or less resistant to tumor development following repeated exposures to PAHs, while other strains are more sensitive or resistant when tested in protocols where an initiating dose of a PAH is followed by repeated doses of a promoting agent, such as TPA or croton oil (Wheldrake et al. 1982). These differences in sensitivity between mouse strains can be linked to differences in metabolic capability, the susceptibility to tumor promotion, and differences in the inflammatory response following the application of a promoting agent. Mouse strains that exhibit less metabolic capability to produce the potentially carcinogenic PAH metabolite, i.e., have less aryl hydrocarbon hydroxylase (AHH) activity, or that are less likely to respond to tumor promotion, i.e., less ornithine decarboxylase (ODC) induction, are more resistant to the development of skin tumors following dermal application of PAHs. Strain differences in metabolic capability, response to tumor promotion and the role of inflammation in tumor promotion are discussed in the following sections.

2.1.3.1 Strain Differences in AHH Metabolism

The potential association between the induction of AHH activity and tumor response has been evaluated in mouse skin painting studies conducted with 3-MC, DMBA, or BaP (Kouri et al. 1973; Sato and Tomita 1998; Watanabe et al. 1975). Kouri et al. (1973) noted that induction of AHH was only present in certain inbred strains of mice, and that these AHH-inducible strains of mice were more susceptible to PAH-induced subcutaneous tumor formation. Kouri et al. (1973) noted further that the inductive potential of AHH was the result of a single autosomal dominant gene. Using backcross progeny of the C57BL/6 (sensitive and inducible) and DBA/2 (resistant and noninducible) mouse strains, Kouri et al. (1973) reported that backcross progeny that were either heterozygous or homozygous for the Ah^{b} (inducible) allele were much more susceptible to 3-MC (150 µg/s.c.) induced tumors (38%-83% tumor incidence) than were the backcross progeny that were homozygous for the Ah^{d} (noninducible) allele (7%-9% tumor incidence). The correlation between AHH induction and 3-MC tumor formation was reported to

be significant ($P < 0.025$), and the data suggested a relationship between AHH induction and 3-MC induced skin tumors.

Sato and Tomita (1998) also reported that the susceptibility of different mouse strains was dependent on the inductive potential of AHH activity in each strain of mouse. Sato and Tomita (1998) evaluated the relationship between AHH induction and carcinogenicity and DNA damage caused by DMBA using BALB/c (inducible), C57BL/6 (inducible), and DBA/2 (noninducible) mice. DNA damage was assessed in single cells (blood) before and after (12, 24, and 36 hours) exposure to 100 mg DMBA/kg (i.p.). Two-stage carcinogenicity assays were conducted, where each mouse received a single dermal application of DMBA (100 µg), followed by croton oil (15 Fg/3 times weekly) for 104 weeks. Maximum DNA damage was observed 12 hours after exposure to DMBA in all strains (Sato and Tomita 1998). The inducible strains of mice (BALB/c and C57BL/6) had significantly more DNA damage than did the noninducible DBA/2 mouse strain, and thus, the authors concluded that AHH induction and metabolism played a significant role in DMBA-induced DNA damage. Further, the inducible strains of mice (BALB/c and C57BL/6) were significantly more sensitive in the two-stage carcinogenicity assay when compared to the DBA/2 strain. Tumor incidence was 50% in BALB/c (15/30), 60% in C57BL/6 (18/30), but only 20% in DBA/2 (6/30) mice.

Watanabe et al. (1975) reported on the relationship between AHH activity and BaP-induced tumor formation. AHH activity was measured in the skin of C3H/He and DBA/2 mice in the presence or absence of 7,8-benzoflavone. Application of 7,8-benzoflavone, an AHH antagonist, had no effect on AHH activity in the skin of DBA/2 mice; however, it decreased AHH activity in the skin of C3H/He mice. Chronic topical application of BaP caused tumors in both strains of mice, but when 7,8-benzoflavone was co-administered with BaP, tumor formation was inhibited only in the C3H/He strain, the same strain in which 7,8-benzoflavone inhibited AHH activity. Thus, as was seen with 3-MC and DMBA, tumorigenesis with BaP also appears to be dependent on AHH activity. Therefore, the inductive potential of AHH for a given species can in part predict the susceptibility of that species to the carcinogenic effects of PAHs.

2.1.3.2 Strain Differences in Response to Tumor Promotion

Two-stage initiation/promotion assays have been conducted in numerous strains of mice, and one strain of mice, the SENCAR strain, was specifically bred to be extremely sensitive to two-stage initiation and promotion (Slaga 1986). Skin tumors have been shown to form in SENCAR mice following the application of TPA without prior initiation and it has been suggested that a small population of epidermal cells in the SENCAR strain are constitutively initiated (Hennings et al. 1981; Yuspa et al. 1982). Thus, the SENCAR strain is highly sensitive to two-stage skin carcinogenesis. However, when tested using a similar two-stage protocol, the tumor incidence in C57BL/6 mice was much lower, i.e., C57BL/6 were essentially resistant to the formation of skin tumors. Thus, there was an obvious strain difference in response to TPA promotion, and the sensitivity of these strains to the two-stage induction of skin tumors has been reported to be SENCAR >> DBA/2 $ C3H/He > BALB/c > C57BL/6 (Angel and DiGiovanni 1999). As discussed in Section 2.4.2.2, tumor promotion in mouse skin involves a complex series of biochemical events that consists of the activation of protein kinase C and the subsequent induction of ornithine decarboxylase (ODC) activity. Thus, any strain differences along this pathway could potentially be responsible for the observed differences in response to TPA tumor promotion. However, the results of studies where these potential differences were investigated indicated that there were no differences in receptor number or affinity in sensitive SENCAR mice compared to resistant C57BL/6 mice (Yamamoto et al. 1988), and ODC induction occurred in sensitive and resistant stains of mice following the application of TPA (Fischer et al. 1989; Kennard et al. 1995; Yamamoto et al. 1988).

Recently, investigators have reported that susceptibility to TPA-induced tumor promotion is a multigenetic trait (Angel et al. 1997; Angel and DiGiovanni et al. 1999). The results of genome scans using promotion-resistant (C57BL/6) and sensitive strains (DBA/2 and C3H) have indicated that sensitive strains possess a TPA susceptibility locus (Ps11) that maps to mouse chromosome 9. This locus is not in resistant strains. Thus, mice that develop skin tumors in the two-stage initiation/promotion likely express this TPA susceptibility gene, and following the application of TPA, the biochemical cascade of events associated with tumor promotion are activated.

Although strains such as C57BL/6 are generally considered resistant to TPA tumor promotion, skin tumors may form in this strain with repeated application of high doses of TPA to

initiated skin (Fischer et al. 1989; Sato and Tomita 1998). Repeated applications (3 or 5 times/week) of TPA (4 Fg) to DMBA-initiated C57BL/6 mice resulted in sustained hyperplasia, ulcerative dermatitis and a tumor incidence that approached 100% after 20 weeks of treatment (Fischer et al. 1989). In mice that received TPA (4 Fg) twice weekly, hyperplasia was not reported and tumor incidence was only 30%. In the Sato and Tomita (1998) study, an increase in skin cancer incidence was observed in C57BL/6 mice that received a single dermal application of DMBA (100 µg), followed by repeated applications of a high dose (15 Fg/3 times weekly) of croton oil, which contains TPA, for 104 weeks. Although the authors do not provide any information on the responses observed in the skin other than skin cancer incidence, it is possible that the high doses of croton oil resulted in an inflammatory response in these mice. These data indicate that even in strains of mice that are resistant to the promotion of skin tumors by TPA, such as the C57BL/6 strain, formation of skin tumors may be observed following repeated application of high doses of TPA that results in inflammation and cytotoxicity and the associated cellular repair processes .

2.1.4 Summary of Bioassay Data

The animal bioassays reviewed are summarized in Table 2.1-8. When considered together, these studies illustrate the following.

1. These studies provide clear evidence that mixtures derived from coal tar are carcinogenic in mice producing significant increases in skin tumor incidence when chronically administered by dermal application.

2. The animal data reported by Warshawsky et al. (1993) and Fraunhofer (1997) provide clear evidence that the potential carcinogenicity of coal tar-derived mixtures is entirely dependent on the constituents in the mixture and cannot be estimated based on BaP content alone. The data in the Warshawsky et al. (1993) study illustrate the importance of the unique chemical composition of a particular complex mixture, and the resulting biological interactions that result from a particular mixture with a specific composition. BaP (0.001%) co-administered

with the weak carcinogen chrysene (0.1%) resulted in a 3.2-fold increase in the incidence of skin tumors (23% incidence), when compared to the effect of chrysene or BaP given alone (7% and 0%, respectively). A mixture of the noncarcinogenic PAHs with the weak carcinogen, chrysene (0.1% each), administered topically also induced tumors in 23% of the mice exposed. Addition of BaP (0.001%) to the mixture of the above PAHs resulted in only a 2-fold increase in the incidence of skin tumors, when compared to the mixture alone. The authors of the paper noted that "the interactions of individual PAHs with BaP vary depending on the PAHs studied," and suggested that the differences in carcinogenic potential between mixtures were likely due to specific interactions between the PAHs at the level of metabolism, DNA adduct formation, or DNA adduct repair. Warshawsky et al. (1993) concluded that the potency of a PAH mixture could not be predicted by the BaP content alone.

The conclusions drawn from Warshawsky et al. (1993) are supported by the results of a 2-year feeding study conducted by Goldstein et al. (1998). This study compared the tumorigenic potential of several coal tar mixtures with a reconstituted mixture in which the BaP content was matched. The authors reported differences in the pattern of tumor formation in mice ingesting coal tar compared to those that ingested BaP, in that coal tar produced tumors of the stomach, liver and lung, whereas BaP alone produced tumors of the tongue, esophagus, and forestomach. Further, intraperitoneal injection of some coal tar mixtures, including a reconstituted mixture of the active coal tar mixture (matched for BaP content), did not result in the formation of tumors in mice, whereas other coal tar mixtures did. Goldstein et al. (1998) concluded that the BaP content alone did not account for the observed tumor incidence, and that the coal tar itself, rather than either the BaP content or the content of carcinogenic PAHs (expressed as BAP equivalents), should be considered the relevant dose-metric.

The data reported by Grimmer et al. (1984) and NIOSH (1979) provide evidence that it is the PAH fraction in the coal tar that is responsible for its activity. Specifically, Grimmer et al. (1984) demonstrated that the carcinogenic

potential of a coal tar mixture was almost entirely attributable to the fraction containing PAHs with three or more rings, with little or no activity attributed to PAC-free fraction. The fraction containing PACs with two and three rings had no activity and the nitrogen-PAH fraction demonstrated little activity. NIOSH noted that of the subfractions tested, the two with the highest concentration of four- and five-ring PAHs possessed the highest biological activity.

3. The data reported in the Fraunhofer (1997) study suggest that the nonneoplastic effects, particularly tissue damage (ulceration) and hyperplasia, were associated with the inflammatory/irritating properties of constituents in creosote and may have contributed to the carcinogenic activity of this PAH mixture. Although ulceration and hyperplasia were observed with both coal tar distillates (CTP1 and CTP2), the severity of these lesions was greatest with CTP2, the distillates where increases in skin cancer incidence were observed following application. Further, both the number of animals diagnosed with one or more of these noncancer lesions as well as the severity of the lesions increased in a dose-dependent manner. Therefore, it is likely that this creosote distillate induced tissue damage, with subsequent regeneration and repair that contributed to the carcinogenic potency of the mixture.

4. The data clearly indicate that differences exist in sensitivity in mouse strains to the same PAH that can be linked to both the metabolic capability of the mouse strain as well as the susceptibility of that mouse strain to tumor promotion. Mouse strains that exhibit less metabolic capability to produce the potentially carcinogenic PAH metabolite, i.e., has less AHH activity, or that are less likely to respond to tumor promotion, i.e., less ODC induction, are more resistant to the development of skin tumors following dermal application of PAHs. However, in strains of mice that are typically resistant to two-stage carcinogenesis (initiation and TPA promotion), a tumor response may be forced with repeated applications

of high doses of TPA that results in inflammation and cytotoxicity and the associated cellular repair processes.

In summary, no adequate studies evaluating the carcinogenic potency of pharmaceutical grade coal tar were identified in a review of the literature. Of the bioassays located that evaluated the carcinogenicity of coal tar-derived mixtures, such as creosote, pitch, and oils, the study conducted with coal tar distillates (creosote) at the Fraunhofer Institute of Toxicology (1997) was the only study with dose-response data. It is important to note, however, that the constituents in creosote and pharmaceutical grade coal tar differ (Lijinsky et al. 1963). Pharmaceutical grade coal tar contained more of the large-ringed PAHs when compared to creosote; however, creosote contained more of the low molecular weight PAHs that are skin irritants. The differences in PAH, as well as non-PAH, constituents can lead to differences in biological effects. Consequently, use of data from a creosote study, which is a completely different mixture, will not provide reliable quantitative estimates of the potency of a pharmaceutical grade coal tar for humans.

2.2 Overview of Epidemiology Studies

There have been anecdotal reports in the literature (i.e., case studies) of individuals with skin tumors who had been occupationally exposed to coal- and coal tar-derived materials or exposed to pharmaceutical grade coal tar as part of a treatment regimen for a skin condition (Annamalai et al. 1981; Dodd 1993). These reports, along with the findings in animal studies (see Section 2.1), have led investigators to conduct epidemiological studies where skin cancer incidence in coal tar-exposed cohorts was evaluated. Cohorts evaluated in these studies included groups that had been exposed to coal tar as treatment for dermatological conditions (i.e., psoriasis or dermatitis) or that were exposed occupationally.

2.2.1 Overview of Epidemiology Studies for Occupational Exposure to Coal Tar

The body of evidence that suggests occupational exposures to constituents in coal tar and other PAH mixtures results in an increase in skin cancer incidence is predominantly from early anecdotal and case study reports (IARC 1984). In 1775, Percival Pott reported cases of scrotal

cancer in individuals employed as chimney sweeps in England (Pott 1775). Following this report, cases of scrotal cancer and skin cancer at other sites, including the head, neck, arms, and hands, were reported in workers employed in other industries including workers in coke ovens, tar distillation, tar and paraffin manufacturing, roofing, creosoting, coal gasification, the cotton industry (mule spinners), and aluminum and steel smelting. As described in the following sections, the majority of patients presenting with skin cancer had two features in common: 1) employment in an occupation where the potential for exposure to PAHs was high, and 2) some form of chronic skin irritation, either associated with exposure to high concentrations of PAHs or with other sources of irritation such as friction from clothing or phototoxicity from repeated exposures to ultraviolet (UV) sunlight. The findings of the occupational studies are briefly summarized in the following section and in Table 2.2-1. A more detailed discussion of the occupational studies and the findings reported in these studies are provided in Appendix A.

2.2.1.1 Studies Evaluating Exposure to Soot

Several case reports have been published that describe cases of skin cancer in workers who were exposed to coal tars or other PAH-containing materials. The early evidence that suggested that occupational exposure to constituents in coal tar and other PAH mixtures results in skin cancer was from early accounts of scrotal cancer cases observed in chimney sweeps employed in England (Pott 1775). However, in the English chimney sweeps, scrotal cancer was generally coupled with other factors, such as poor hygiene and cell injury resulting from chronic irritation (Butlin 1892). According to Butlin (1892), a sequence of symptoms was present in the chimney sweeps, with a general progression beginning with irritation, inflamation, dermatitis and folliculitis of the scrotum and surrounding groin area, followed by the formation of “soot” warts with keratotic horns on the scrotum, some of which upon further irritation, progressed to ulcerated epithelioma.

In contrast, scrotal cancer was rarely, if ever, seen in chimney sweeps employed in other countries, such as Belgium, Germany, and France (Butlin 1892). The reasons cited for the lack of scrotal cancer in other areas of the world were that the clothing worn by other chimney sweeps was more protective and that chimney sweeps in other countries practiced good hygiene. In comparing the attire, cleanliness, and living quarters between the English sweeps and those

employed in Belgium, Germany, and France, Butlin (1892) reported that for English chimney sweeps, the clothes were made of a thick material, but were dirty and at times torn and tattered. The sweeps took baths at most once per week, and the living quarters were extremely dirty with a high presence of soot. In contrast, sweeps of other countries wore similar clothing, but they usually cleaned their clothes once or twice per week or had more than one set of work attire. Many of the sweeps in Belgium and Germany wore hoods accompanied by a face wrap instead of a normal cap. Sweeps in these countries practiced better hygiene, with some taking two baths per day, one in the shop after work, and another at home. And lastly, the living quarters of the sweeps in countries other than England were clean and free of soot. Consequently, the extent and duration of soot exposure were clearly different. Further, the practice of good hygiene not only decreased exposure by removal of the soot from the skin, but also decreased the irritation associated with continuous presence of soot on the skin. Therefore, irritation from the presence of soot appeared to play a critical role in the development of scrotal cancer in sweeps.

The most recent epidemiology study conducted for chimney sweeps evaluated the incidence of cancer in a cohort of 5,242 chimney sweeps employed between 1957 and 1987 through the national trade union in Sweden (Evanoff et al. 1993). The average years of employment were 12 ± 12.2 years with a median employment of 9.0 years. No increase in the incidence of skin cancer was found (observed = 4; expected 6.5; SIR = 0.61). As an explanation for the lack of skin cancer in this cohort, the authors of the study noted that Swedish sweeps have long had the right to bathe on paid time following work. Thus, hygiene practices were likely better in this cohort than in the English cohort.

2.2.1.2 Studies Evaluating Exposure to Mineral and Cutting Oils

In addition to chimney sweeps and coal tar workers, scrotal cancer has been observed in workers exposed to mineral oils in the processing of cotton (cotton mule spinners). As presented by Hoffman (1928), of 74,625 men employed as cotton mule spinners between 1911 and 1920 in England and Wales, 100 developed scrotal cancer. Hoffman (1928) concluded that the incidence of scrotal cancer in cotton mule spinners was due to a combination of exposure to PAHs present in the lubricating oils and chronic chemical and mechanical irritation from the trousers. The importance of the mechanical irritation was highlighted by the fact that although

wool mule spinners were exposed to PAHs to the same extent as cotton mule spinners, no reports of scrotal cancer had been reported in wool mule spinners. Hoffman (1928) noted that the explanation for the differential incidence rate was that cotton mule spinners operated in a hot, moist environment that predisposed the employee to irritation from the trousers, whereas the wool mule spinners operated in a cool, dry environment. Hence, in addition to the potential contribution of exposure to PAHs, as with the British chimney sweeps, irritation may have been a factor.

In a study conducted by Waldron et al. (1984), the incidence of scrotal cancer reported in the West Midland Regional Cancer Registry between 1936 and 1976 was evaluated according to occupation. A total of 344 cases of scrotal cancer was registered, of which 61% occurred in individuals exposed to mineral oils. The incidence of scrotal cancer in individuals with no known exposure to PAHs was 16.6%. These data qualitatively illustrate the occurrence of scrotal cancer in individuals exposed to mineral oil and cutting oil, as well as in individuals who were not. No quantitative interpretation could be made because the number of individuals in each group (exposed vs. nonexposed) was not reported. Although these data do suggest that an increased incidence of scrotal cancer was associated with exposure to lubricating oils, the authors noted that poor hygiene was likely a contributing factor in the formation of the scrotal tumors.

2.2.1.3 Studies Evaluating Exposure to Coke Oven Tar, Creosote or Pitch

In a number of occupations workers were potentially exposed to coal tar, coal tar fumes, creosote, or pitch dust or fumes including workers at facilities such as gas-works, coal tar distilleries, coke ovens, creosote plants, as well as roofers. The earliest reports of the occurrence of skin cancer in coal tar workers consisted of individual case reports describing cases of scrotal cancer (Bell 1876; Butlin 1892; Volkman 1875). However, Hoffman (1928) noted that scrotal cancer was predominately reported in individuals employed in industries where the potential for irritation was high and that irritation played a role in the formation of scrotal cancer. Thus, as with the chimney sweeps discussed in the previous section, irritation was a factor in the formation of scrotal tumors. In a more recent study, conducted by Waldron et al. (1984), the incidence of scrotal cancer reported in the West Midland Regional Cancer Registry between 1936 and 1976 was evaluated according to occupation. Only 7.9% occurred in individuals

exposed to coal tar or pitch, while 16.6% of scrotal cancer were reported in individuals with no known exposure to PAHs.

Cases of skin cancer have been reported at sites other than the scrotum in workers in the coal tar industry, with the early evidence based on case reports of workers employed in coal tar distillation facilities (Ball 1885; Ross 1948). In contrast to the early reports from Britain, Heller (1930) suggested that skin cancer resulting from exposure to coke oven tar or coal tar at tar distilleries in the U.S. was low with no cases of skin cancer reported in several coal tar distillation, coke oven, and coal gasification facilities. These findings were consistent with those of Wood (1929).

There were, however, early reports that describe cases of skin cancer in individuals employed in coal tar industries in the U.S. (Heller 1930; Schamberg 1910). As with the reports from England, for the tumors on sun-exposed areas, exposure to UV-B irradiation was not taken into account in the studies of cohorts in the U.S. Further, data regarding exposures to coal tar were not presented. However, attempts were made to classify the incidence of skin cancer by occupation and job position (Heller 1930).

More recent epidemiological studies have evaluated the skin cancer incidence in cohorts of workers in the U.S. as well as other countries who were employed in industries with the potential for exposure to coal tar, pitch and pitch volatiles using skin cancer incidence rates in control populations for comparison. In a study by Sexton (1960), the incidence of skin cancer in a group of 359 workers at a coal hydrogenation plant in West Virginia was evaluated. A total of five cases of skin cancer were reported, including four cases of squamous cell carcinoma (SCC) and one case of basal cell carcinoma (BCC). The incidence of skin cancer in this cohort was reported to be significantly increased ($p = 0.032$) when compared to a background incidence rate (Sexton 1960). However, it is important to note that four of the five cases were located on the head, neck or hand, which are sites commonly associated with tumors caused by UV light (Slaper and van der Leun 1987).

A recent study conducted in Germany by Letzel and Drexler (1998) evaluated the incidence of skin cancer in 606 workers who were exposed to coal tar, pitch and distillate products and were recognized as having occupationally-induced disease between 1946 and 1996. There were 380 SCCs in 151 individuals and 218 BCCs in 98 individuals identified in the study.

Although the authors claim that the role of UV light in this cohort was over-valued, more than 90% of the SCCs and BCCs were located in sun-exposed areas. Thus, there is a high probability that UV light did contribute to the formation of skin tumors in this cohort. Letzel and Drexler (1998) note that because of methodical reasons, the exact quantification of the incidence of skin tumors in this cohort was not possible. More specifically, there was no control group suitable for comparison because of the year of birth range (1882-1960).

Sherson et al. (1991) evaluated the cancer incidence in a cohort of 6,144 male foundry workers who had been invited to participate in x-ray examinations in Denmark as part of a national silicosis survey. The materials to which that the workers were exposed were not reported; however, coke ovens are typically located at foundries, so these workers may have been exposed to high-temperature coke oven tar. The cancer incidence rates were compared to expected values for the Danish population. The standard morbidity ratio (SMR) for nonmelanoma skin cancer was 0.93 (65 cases observed/69.86 cases expected), and thus, the authors reported that there was no significant increase in the incidence of skin cancer in this cohort of foundry workers, when compared to the general population.

Recent studies evaluating the incidence of skin cancer in aluminum workers suggest that the risk of skin cancer resulting from exposure to coal tar pitch volatiles (CTPV) is very low. Spinelli et al. (1991) reported on the incidence of nonmelanoma skin cancer in 4,213 aluminum workers employed in British Columbia between 1954 and 1985, with comparisons made to the expected skin cancer incidence reported by the British Columbia Cancer Registry. The Standardized Incidence Ratio (SIR) for nonmelanoma skin cancer was only 0.51 (38 cases observed/74.31 cases expected = 0.51). Thus, the risk of nonmelanoma skin cancer in this cohort of aluminum workers was half that expected for the general population.

A study by Rønneberg and Andersen (1995) reported on the mortality resulting from nonmelanoma skin cancer in 1,137 workers employed at an aluminum smelter between 1922 and 1975. Comparisons were made to the expected incidence in the general population. The SIR (observed/expected) for skin cancer in workers employed for less than 3 years was 3.09. However, for workers employed for 3 or more years, the SIR for skin cancer was only 0.79. Thus, there was no increased risk of skin cancer associated with long-term employment at the aluminum smelter.

Skin cancer was evaluated in a cohort of 5,939 roofers exposed to coal tars and pitches, and bitumens (Hammond et al. 1976). Exposures were reported to range from 14 $\mu g/m^3$ in the roof-tarring area to 6,000 $\mu g/m^3$ around the coal tar roofing kettle area. However, the positions for the individuals who died with skin cancer were not identified. Five deaths resulting from skin cancer were observed as opposed to 1.18 expected deaths and the SMR for skin cancer in this cohort was reported to be greater than 4.0. Thus, the authors concluded that the number of deaths resulting from skin cancer in this cohort was significantly above the expected value for the U.S. male population. However, the study authors did not map the skin tumors to anatomical region and the potential contribution of UV light was not considered.

The potential skin cancer risk in a cohort of 922 workers who worked as creosote impregnators and were exposed to creosote regularly was evaluated by Karlehagen et al. (1992). The exposures were poorly characterized; however, the authors noted that all of the workers were exposed to naphthalene, a component of creosote. A marginally statistically significant increase in the incidence of nonmelanoma skin cancer was reported (9 cases observed versus 3.79 expected; SIR = 2.37), with most of the cases (6) reported in the Swedish workers. The authors noted that comparisons were made to national cancer rates, which could have introduced bias into the study because the base risk of cancer differed between urban and rural areas where the plants were located. The authors further noted that the workers worked partly outdoors and were exposed to UV light, which likely contributed to the incidence of skin cancer.

A characteristic common to occupations where an increased incidence of skin cancer was associated with PAH exposure was concomitant acute and chronic skin irritation. This irritation took the form of burns (tar or pitch burn), erythema and edema, dermatitis, folliculitis, pigmentation, warts, hyperplasia (shagren skin), and ulceration. Additionally, the working conditions encountered were often hot, inducing perspiration, thus leading to chapping of the skin due to friction resulting from clothing. Hoffman (1928) concluded that irritation played a major role in the carcinogenic process leading to scrotal tumors in cotton mule spinners. Fisher (1953) found a significant correlation between coal tar-induced erythema and the occurrence of tar warts in tar distillation workers. Lueke (1907) reported that the skin of carbon workers became chronically inflamed, followed by moist eczema accompanied by inflamed hair follicles, acne form eruptions, and the formation of crust and scales. Ross (1948) reported that tar and

pitch burns, erythema, folliculitis, acne, comedones, dermatitis, hyperplasia and melanosis were all present in tar and pitch workers. Waldron et al. (1984) noted that in one of the cases of scrotal cancer in an individual with no known exposure to PAHs, poor hygiene was a likely contributor in the development of cancer. Lastly, all of the early epidemiology studies commented on the irritating effects of soot, coal tar, paraffin, pitch, and oils to skin, as well as mechanical irritation resulting from scratching warts that are predisposed to itch.

In addition to the direct irritating effects of coal tar and pitch, exposure to the volatile and semi-volatile chemicals present in coal tar could lead to a photosensitization, thus, increasing the erythema induced by sunlight. Approximately 90% of all skin tumors identified in workers at coke ovens, coal gasification facilities, and coal tar distilleries were located on the head, neck, arms, or hands (Heller 1930). These were also the areas associated with increased skin cancer resulting from exposure to UV light (Slaper and van der Leun 1987), which is important because the potential contribution of UV light to skin cancer incidence was not considered in the occupational studies reviewed.

In summary, various studies over the last 200 years have illustrated the occurrence of skin cancer in workers employed in occupations where the potential for exposure to PAHs was high. In most instances, however, the prevalence of skin cancer in a particular occupation could also be linked to some characteristic that facilitated the formation of tumors, such as irritation, exposure to UV light, or skin conditions resulting from poor hygiene (ATSDR 1996).

2.2.2 Overview of Epidemiology Studies for Medicinal Use of Coal Tar-Containing Products

In an FDA-convened symposium, the incidence of skin cancer in patients treated for psoriasis with coal tar at several institutions was reviewed, and it was concluded that the evidence did not support any association between the use of coal tar-containing products and the development of skin cancer (Pittelkow et al. 1981). Further, the National Psoriasis Foundation reviewed the medical histories of 15,000 of its members and also found no suggestion of an increased risk of skin cancer in patients who used coal tar-containing products (Pittelkow et al. 1981). Since those surveys, there have been one prospective and several retrospective or case control epidemiological studies that have considered the association between treatment regimens

for psoriasis and skin cancer. While there have been reports of an increase in the incidence of skin cancers in patients treated for psoriasis using a variety of other treatments, when those patients who had received only coal tar therapy were evaluated or when the effects of other treatments were controlled, there has been no clear evidence of an increase in the incidence of skin cancer or systemic cancer in patients, when compared to reference populations (Jones et al. 1985; Maughan et al. 1980; Pittelkow et al. 1981; Stern and Laird 1994; Stern et al. 1998). These studies are summarized in Table 2.2-2.

2.2.2.1 Comparison of Psoriatic and Normal Skin

As noted, pharmaceutical grade coal tar has been used for decades to treat patients with psoriasis. Coal tar-containing shampoos have also been prescribed for patients with psoriasis and dermatitis but are also available as over-the-counter shampoos that may be used by persons with a variety of skin conditions. Epidemiological studies have been conducted to evaluate the potential for skin cancer and systemic cancers in patients treated with pharmaceutical grade coal tar-containing ointments for psoriasis (Grupper and Berretti 1980; Jones et al. 1985; Menter and Cram 1983; Pittelkow et al. 1981; Stern and Laird 1994; Stern et al. 1998) or for atopic dermatitis (Jemec and Østerlind 1994; Maughan et al. 1980). Skin cancer incidence in psoriasis patients treated with coal tar has also been compared to the incidence of skin cancer in the general population (Alderson and Clark 1983; Bhate et al. 1993; Halprin et al. 1982 Stern et al. 1985).

It had been postulated that psoriasis patients, due to the nature of the skin disease, were less susceptible to the development of skin cancer as a result of exposure to coal tar. This was initially proposed by Chapman et al. (1977), who reported that the activity of AHH was lower in psoriatic skin than in uninvolved skin of psoriasis patients and than in normal skin. These data were not supported by other experimental evidence (Bickers et al. 1984; Bickers and Kappas 1978; Lawrence et al. 1984) and was retracted by the laboratory that conducted the investigation (Finnen et al. 1983).

An evaluation was conducted to compare key parameters that would affect either the amount of exposure (e.g., absorption) or the potential for a response (e.g., metabolism) in the skin of psoriasis patients to skin for persons without psoriasis. With regard to absorption, a metabolite

of one of the constituents in pharmaceutical grade coal tar, pyrene, has been used as an indicator of absorption in persons exposed to pharmaceutical grade coal tar in psoriasis patients (Santella et al. 1994) and in volunteers (Van Schooten et al. 1994). As discussed in more detail in Section 3.0, 1-hydroxypyrene (1-OH-P) was recovered in the urine of patients following application of coal tar ointment for the treatment of psoriasis and in the urine of volunteers using a coal tar-containing shampoo, indicating that absorption of constituents in coal tar had occurred in both populations. The specific quantitative relationships between application and absorption for these two exposure routes are discussed in further detail in Sections 3.0 and 4.0.

Absorption of other compounds in normal and psoriatic skin has been investigated. Dermal penetration of anthralin, another drug used to treat psoriasis, measured *in vitro* was significantly greater in active involved psoriasis skin than in uninvolved psoriasis or normal skin (Wang et al. 1987). Large variations in dermal penetrations of anthralin were found among psoriatic skin samples, leading the authors to suggest that dermal penetration depended on the disease state of the psoriasis, with penetration increasing with increased severity. The concentration of another agent used to treat psoriasis, the steroid, triamcinolone actonide, was 3 to 10 times higher in the epidermis and dermis of involved psoriatic skin than in normal skin, as measured by the dermal penetration of radiolabeled material from an ointment or cream applied *in vitro* (Schaefer et al. 1977). In contrast, no significant differences were observed in the percent of the applied amount of hydrocortisone in psoriatic patients (2.32%) compared to normal volunteers (2.45%) (Wester et al. 1983). The authors concluded that hydrocortisone absorption was the same in normal skin as in “presumably stable psoriatic plaques.” These data indicate that the potential for dermal absorption is at least as great or greater in persons with psoriasis than in persons without psoriasis.

The comparative ability of psoriatic skin to metabolize constituents in coal tar was considered. Basal AHH activity and epoxide hydrolase activity were the same in the epidermis from age-, sex- and smoking habit-matched nonpsoriatic volunteers and persons with psoriasis (Bickers et al. 1984). Basal activity of AHH was approximately 0.06 pmole 3-hydroxybenzo(a)pyrene (3-OH-BaP)/min/mg protein in both groups and epoxide hydrolase activity was approximately 25 pmol BaP 4,5-diol/min/mg protein. Following induction with BA, AHH levels increased by 165% in the skin samples from patients without psoriasis and 320% in

the skin of patients with psoriasis. Basal and inducible levels of AHH activity were the same for patients with psoriasis and those with atopic dermatosis (Bickers and Kappas 1978).

Further, total metabolism of BaP by epidermal microsomes and the overall pattern of metabolites formed were similar in patients with psoriasis (202 pmol metabolites/60 min/mg protein) and in volunteers (215 pmol metabolites/60 min/mg protein) (Bickers et al. 1984). Moreover, the formation of the 7,8-diol-BaP metabolite was only slightly higher in psoriatic skin compared to normal skin (4.8 pmol metabolites/60 min/mg protein compared to 3.2 pmol metabolites/60 min/mg protein). These data indicate that the ability to metabolize constituents in coal tar is similar in normal and psoriatic individuals.

A comparison of the production of DNA adducts in normal human skin and psoratic skin has been conducted (Phillips et al. 1990; Schoket et al. 1990). In this study, DNA adduct levels in skin biopsies from hospitalized patients undergoing standard coal tar treatment (amount of coal tar not specified) for psoriasis were obtained and compared to normal human skin explants to which a 1.5% coal tar ointment was applied. [A single application of coal tar at 90-105 µg coal tar/cm^2 skin was applied to normal skin. As a comparison, if psoriasis patients were treated using the standard Goeckerman protocol, then application of a 2% coal tar ointment would result in approximately 100-150 µg coal tar/cm^2 skin, assuming whole body coverage.] The number of DNA adducts, expressed as fmol/µg DNA, was similar (0.19 in normal skin and 0.22 in psoriatic skin) (Phillips et al. 1990). A comparison of DNA repair capability has also been studied (Dybdahl et al. 1999). In this study, the DNA repair capacity in lymphocytes from psoriasis patients with and without BCC was compared to a control group who also did or did not have BCC. DNA repair rates in psoriasis patients without BCC were not significantly different than in their corresponding age- and sex-matched normal controls. When psoriasis patients were divided into groups of high and low repair rates, those with skin cancer had lower DNA repair rates than psoriasis patients without skin cancer. This same relationship was observed among the individuals without psoriasis.

As discussed in detail in Section 2.4, induction of ODC activity is a critical step in the induction of DNA synthesis and is a key event in tumor promotion. Arnold et al. (1990) measured ODC activity in the skin of normal, healthy volunteers and from the uninvolved skin of psoriasis patients at various time points after tape stripping (tape stripping simulates skin

"wounding"). There were no differences in ODC response between the nonpsoriasis group and the psoriasis patients. Application of pharmaceutical grade coal tar for 16 hours to involved and uninvolved skin of psoriasis patients or to the skin of normal volunteers followed by tape stripping of those areas did not significantly alter ODC activity (a 21% decrease was noted) compared to that measured with tape stripping of skin not treated with coal tar ointment (Arnold et al. 1993). Further, no difference was noted in ODC induction between patients with psoriasis and normal volunteers even when the duration of coal tar treatment was increased to twice daily applications for one week (Arnold et al. 1993). These data indicated that no difference in a key pathway in tumor promotion exists between patients with psoriasis and persons without psoriasis, providing evidence that both would be able to respond to an agent that could cause skin cancer. However, application of pharmaceutical grade coal tar did not induce an ODC response.

Clinical studies, discussed in Section 2.2.2.4, provide evidence that psoriasis patients not treated with PUVA developed skin cancer at the same frequency as individuals with normal skin (Alderson and Clarke 1983; Bhate et al. 1993; Stern et al. 1985). Patients with psoriasis treated with PUVA did have an increase in the incidence of SCCs (Stern and Laird 1994; Stern et al. 1998), indicating that psoriatic skin is capable of responding to carcinogenic agents.

When absorption, enzyme activation, metabolism, and the potential for increases in DNA synthesis are considered, no differences in responses for psoriatic skin compared to normal skin were noted. As stated, clinical studies support the lack of difference in skin cancer incidence in these populations. Consequently, conclusions drawn with regard to the potential effects of use of pharmaceutical grade coal tar from epidemiological studies of psoriasis patients treated can be applied to persons without psoriasis.

2.2.2.2 Epidemiology Studies of Psoriasis Patients Who Received Coal Tar Therapy

Psoriasis is a persistent skin condition that has been treated with multiple regimens, some of which involve the application of coal tar-containing products as part of the therapy (Stern 1995). Several studies have been conducted where the incidence of skin cancer was evaluated in cohorts that were treated with coal tar for psoriasis (Jones et al. 1985; Pittelkow et al. 1981; Stern and Laird 1994) (Table 2.2-2). A common therapeutic regimen for psoriasis that includes the use of coal tar is the Goeckerman regimen that was initially described by Goeckerman in 1925

(Menter and Cram 1983; Muller and Perry 1984; Perry et al. 1968). All of the epidemiology studies for psoriasis patients treated with coal tar indicated that the clinical regimen by which these patients were treated was basically the Goeckerman treatment. Since its introduction, the Goeckerman treatment for psoriasis has followed a consistent regimen at the Mayo Clinic and other treatment centers (Menter and Cram 1983; Muller and Perry 1984; Perry et al. 1968).

In this regimen which continues to this day (Silverman et al. 1995), coal tar ointment at concentrations ranging from 2% to 5% is applied daily to all areas of the body, except the face, genitalia, and axillae. The material is reapplied three times a day or as often as necessary to keep the skin continually covered (Menter and Cram 1983; Muller and Perry 1984; Perry et al. 1968). Thick cotton pajamas are worn by all patients and tar is applied to hands under plastic gloves and feet under cotton socks (Menter and Cram 1983). After 24 hours, the excess tar is wiped with a gauze pad saturated in oil; the patient is then exposed to generalized minimal-erythema doses of UV-B light, bathed with ordinary bar soap, and fresh tar reapplied for the next 24 hours (Menter and Cram 1983; Muller and Perry 1984; Perry et al. 1968). The scalp is shampooed daily with a coal tar shampoo followed by application of a 10% liquor carbonis detergens to the scalp. Plastic shower caps are worn all day. To achieve and maintain maximum contact between coal tar and skin, pajamas are not changed until they became heavily impregnated with tar, usually every fifth day (Menter and Cram 1983). Hospital treatment continues for 2-4 weeks (Muller and Perry 1984) with some day-care treatments lasting up to 38 days (Menter and Cram 1983).

Remission with this treatment was limited with median remission times ranging from a few months to up to a year or longer. Consequently, at-home treatment continued for some patients for years (Maughan et al. 1980; Muller and Perry 1984; Pittelkow et al. 1981). Maughan et al. (1980) stated that their patients used CTPs ranging from no days after hospitalization to daily usage for 26 years. As described in more detail in Section 3.1, the average amount of a 2% coal tar ointment used during hospitalization entailed 3 grams coal tar/day, which would amount to 60 grams applied during a standard 20-day treatment regimen (Muller and Perry 1984). This does not include the amount of coal tar used at home.

Stern et al. (1980), Stern and Laird (1994), Stern et al. (1998)

In a series of studies conducted by Stern and colleagues (Stern et al. 1980; Stern and Laird 1994; Stern et al. 1998), a cohort of roughly 1,380 individuals who received psoralen and ultraviolet radiation A (PUVA) as treatment for psoriasis were followed up to determine if skin cancer effects could be determined. The average follow-up time was 13.2 years in 1994. Each individual was questioned in order to provide data regarding exposures to other agents used for psoriasis therapy, including topical coal tar/UV-B (the use of coal tar highly correlated with UV-B use, i.e., as in the Goeckerman regimen), methotrexate, and ionizing radiation, and the correlation between these treatment modalities and skin cancer incidence was evaluated. The three studies cited provide information specifically about use of coal tar among the PUVA-exposed patients.

Data regarding the average exposure to coal tar were not provided; however, the authors assumed that high exposures to the coal tar/UV-B consisted of 300 UV-B treatments or treatment with coal tar for more than 45 months. The results of a Poisson regression analysis in the latter two reports (Stern and Laird 1994; Stern et al. 1998), which controlled for age, sex and geographic location and level of PUVA exposure, indicated that there were no substantial increases in the incidence of SCC or basal cell carcinoma (BCC) associated with long-term use of coal tar or UV-B. This conclusion was noted by the authors (Stern and Laird 1994) to be in contrast to their earlier findings for this cohort when the cohort had far less exposure to PUVA (Stern et al. 1980). However, there were significant increases in the incidence of SCC associated with PUVA exposures.

It is worth noting that the patients followed by Stern and his colleagues were all treated extensively with PUVA. Therefore, any conclusions about the effect of coal tar/UV-B drawn from these studies are only relevant to those who have also been treated with PUVA. These studies are not relevant to a risk assessment for coal tar exposure in and of itself. Nevertheless, it appears that coal tar/UV-B exposure does not add to the known risk of skin cancer associated with PUVA treatment.

Maier et al. (1996)

Maier et al. (1996) conducted a retrospective study of 496 patients who had PUVA treatments initiated prior to 1987 and who had received more than five PUVA exposures. The median duration of follow-up was 82 months (less than 7 years), where follow-up time was defined as the time from the first PUVA treatment to the date of first nonmelanoma skin cancer or the date of interview with the study authors. In addition to the PUVA exposures, exposures to arsenic, x-rays, coal tar, UV-B, methotrexate, and retinoids were recorded. Note, however, that these exposures were recorded only as "present" or "absent," i.e., no quantitative information on exposure levels was ascertained. Cox proportional hazards regression models were applied to the follow-up time data.

Although Maier et al. (1996) suggest that tar exposure has a significant effect, this conclusion and its relevance to a population of coal tar users are weak. First, the authors performed both marginal analyses (looking at one predictive variable at a time) and partial analyses (looking at all the predictive variables together). Because of the known effects on skin cancer of several of the predictive variables (e.g., arsenic, x-rays, and methotrexate) and the obvious problem of correlation among the exposure variables (the presence of one exposure variable being correlated with another exposure variable for any given individual), the marginal analyses are meaningless. The only way to attempt to ascertain the effect of one of the exposure variables is to adjust for the presence of the others, as done in the partial analyses. The only statistically significant effect of coal tar in the partial analyses is for the combined endpoint (both SCC and BCC), and the p-value associated with that hypothesis test is 0.04, i.e., barely less than a 0.05 level that is commonly considered the "bare minimum" for statistical significance. Indeed, a common statistical practice would be to correct the reported p-value for multiple statistical tests, and there are many tests in this report, in which case the value of 0.04 would be *increased*, probably above the 0.05 level. Coal tar exposure is not significantly related to BCC.

In addition to the issue of the significance of the coal tar variable, there are fundamental problems with interpretation of the results. First, the exposure variables are "present/absent" summaries. There is no way to relate the relative risks reported for coal tar (or any of the other exposure variables besides UV-A) to a coal tar exposure level.

Moreover, the Cox analysis that was performed relates exposure variables to the length of survival without skin cancer *following PUVA treatment*. That is, the relative risks reported have nothing to do with the length of time between coal tar exposure, for example, and development of skin cancer. They are only relevant to an assessment of the consequences of PUVA exposures. The analyses that have been done, therefore, reveal nothing about the effects of the level of tar exposure on skin cancer risk nor do they address the time to development of skin cancer following coal tar exposure.

Similar to the problems associated with the studies by Stern and his colleagues (described above), the conclusions of this study are problematic in the sense that they are not at all relevant to an assessment of coal tar skin cancer risks. What is needed are data sets whose results are not conditional on the presence of extensive PUVA treatment.

Jones et al. (1985)

The skin cancer incidence in a cohort of 719 individuals (305 males and 414 females) who received topical coal tar therapy for psoriasis over a 10-year period between 1953 and 1973 was evaluated by Jones et al. (1985). Psoriasis treatment consisted exclusively of coal tar and none of the individuals had received treatments of UV or ionizing radiation or cytotoxic drugs. The treated population was selected to allow a latent period of at least 10 years and up to 30 years for some patients after coal tar use and the mean ages at the initiation of treatment were 27 and 23 years in males and females, respectively. Thirteen percent of the population was 15 years of age or younger at the beginning of therapy, while 44% were between the ages of 15 and 35, 25% between the ages of 35 and 64 years of age, and 8% were 64 years or older at the beginning of treatment. The expected cancer incidence was calculated using data from the West of Scotland Cancer Registry for a group of the same number of persons and with a similar age and sex distribution. There were no significant increases in the incidence of melanoma or nonmelanoma skin cancer in the coal tar-treated group, when compared with the reference population.

Pittelkow et al. (1981)

Pittelkow et al. (1981) conducted a 25-year follow-up study (average follow-up was 20.1 years) for 260 psoriasis patients who received the Goeckerman treatment (coal tar-based

ointment and UV-B irradiation) for the first time between 1950 and 1954. Medical histories, pre-existing malignant or premalignant conditions, as well as any medical treatments that may be associated with an increased cancer risk, such as the use of arsenicals, methotrexate, or ionizing radiation, were recorded. More than 85% of the patients had severe or generalized psoriasis that required almost total body coverage with coal tar ointment during the therapy. Nineteen of the 260 patients reported nonmelanomatous skin cancers, two-thirds of which were in sun-exposed areas. Of the patients with skin cancer, 56% used coal tar at home, while 72% of the patients without skin cancer used coal tar at home following hospital treatment (based on responses from a subset of the 260 patients). Of the patients with skin cancer who used coal tar at home, 67% of these patients used the coal tar treatment at home for more than 50 days/year. Of the patients without skin cancer who used coal tar at home, 56% used the coal tar treatment at home following hospital treatment for more than 50 days/year. The median duration of at-home coal tar treatment (at least one day/year) in patients diagnosed with cancer was 21 years, whereas patients without skin cancer reported using at-home coal tar therapy for a median of 14 years. However, neither the incidence of skin cancers nor cancer at any other site was increased in this cohort of patients, when compared to the age-specific incidence rates in skin cancer recorded in the Third National Cancer Survey (TNCS) for the United States (Scotto et al. 1974). When considered on a regional basis, the incidence of skin cancer in the patient population was half that expected if the entire population lived in an area where UV irradiation is high, such as the Dallas-Fort Worth area, and similar to that expected if this entire patient population lived in an area of low UV irradiation, such as the Iowa or Minnesota-St. Paul areas. Further, not only was there no increase in the incidence of skin cancer in treated patients, but also, in contrast to patients treated with PUVA as reported by Stern et al. (1980) and Stern and Laird (1994), there was no increase in the ratio of BCCs to SCCs. Pittelkow et al. (1981) concluded that there was no increase in the incidence of skin cancer, or cancer at other sites, in these patients treated with a combination of coal tar and UV-B irradiation. A detailed discussion of this study is given in Section 3.0.

Qualitative, supporting evidence of the safety of coal tar therapy in the treatment of psoriasis has been published for other patient populations treated with coal tar (Grupper and Berretti 1980; Menter and Cram 1983).

Grupper and Berretti (1980)

Grupper and Berretti (1980) reported that they had not observed an increase in skin cancer among more than 1000 patients treated over a 10-year period at the Rothschild Foundation in Paris with "intensified" Goeckerman regimen therapy using 2-5% coal tar along with twice daily UV-B treatment. Grupper and Berretti (1980) also reported that they had treated approximately 4,000 patients (more than 40% of the skin surface involved) at the Foundation Francaise du Psoriasis between 1975 and 1980. While the follow-up time was limited for this group, no increases in skin cancer had been noted at that time in those patients.

Menter and Cram (1983)

Menter and Cram (1983) reported the combined experience at two United States psoriasis centers, University of California Psoriasis Center and Baylor Medical Plaza Psoriasis Center. No increases in skin cancer attributable to treatment were found among 300 patients ranging in age from 3 to 82 years old. These patients had 30-90% of the skin surface area involved and had undergone at least one round of Goeckerman therapy (coal tar and UV-B). Treatment was given during the time period from 1973-1981 and follow-up was for at least 1 year. Some patients required only one course of Goeckerman treatment, while others underwent a once-yearly therapy. However, at-home usage following hospital treatment continued for all 180 patients treated at the University of California. The average duration of at-home coal tar usage was 15.6 years, with 45% of patients using tar therapy more than 5 years but less than 10 years, and an additional 35% of patients using the CTPs for 10 years or more but less than 20 years. BCCs were noted in less than 5% of patients; however, all were on the face (to which coal tars were not applied), all the affected patients were over 60, and in half of these patients, the BCC was present prior to initiation of the coal tar treatment. Coal tar therapy using a modified Goeckerman regimen continues to be part of the treatment modalities used along with PUVA, methotrexate and retinoids at Baylor (Silverman et al. 1995).

2.2.2.3 Studies of Dermatitis Patients Who Received Coal Tar Therapy

This section describes studies that have compared the incidence of skin cancer in patients with nonpsoriasis skin conditions who were treated with coal tar to that in the general public without psoriasis.

Maughan et al. (1980)

Maughan et al. (1980) conducted a 25-year follow-up study of skin cancer in 305 patients with atopic dermatitis and neurodermatitis who were treated with coal tar ointments and UV-B irradiation at the Mayo Clinic between 1950 and 1954. This was a parallel study to that conducted for patients treated for psoriasis (Pittelkow et al. 1981), and was conducted to determine if psoriasis patients were more resistant to skin cancer caused by PAHs than other individuals. The age at first treatment with the Goeckerman regimen ranged from 1 year to late adulthood. Coal tar ointment use in this population after release from the hospital varied from none to everyday use for 26 years. The authors reported that the frequency and duration of coal tar therapy did not differ between those patients who developed skin cancer (11 of 305 patients) and those who did not. Further, the incidence of skin cancer in this population was not significantly increased above the expected incidence of skin cancer (18.8 for the Dallas-Fort Worth area; 9.4 for San Francisco-Oakland; 6.7 for the Minneapolis-St. Paul area, and 5.3 for Iowa) recorded in the TNCS for the United States (Scotto et al. 1974). Many of the patients resided in the southern United States; therefore, the expected values for the Dallas-Fort Worth area would be most relevant for the comparison of observed and expected numbers of cancers. Thirteen patients were diagnosed with skin cancer, with 10 of the 11 nonmelanoma skin cancers being located on the head or neck. Eight patients had noncutaneous malignancies. The expected values for the Dallas-Fort Worth area were reported to be 18.8 for nonmelanoma skin cancer and 16.2 for noncutaneous malignancies. Therefore, the authors concluded there were no increases in the incidences of skin cancer or noncutaneous malignancies associated with the use of coal tar and UV-B irradiation.

Jemec and* Østerlind *(1994)

Patient files at the Department of Dermatology, Rigshospitalet in Copenhagen were reviewed to identify patients with atopic dermatitis who were admitted for treatment in the period 1917 to 1937 (Jemec and Østerlind 1994). All of the patients had been extensively treated with coal tar. Although the specific treatment regimen was not given, it is likely that these patients received treatment that was the same or similar to that administered during the Goeckerman treatment. A total of 117 patients was identified, of whom 88 had sufficient information to be eligible for this study. The mean age of the patients upon admission to the hospital was 19 years. The patients were admitted for an average of 30 days, with some patients receiving more than one course of treatment during the time period. Of these 88 persons, 13 had more than one course of treatment. Among the 88 patients, 3,392 person-years of follow-up were accrued (average number of years of follow-up was 38.5; 37.2 years for men and 40.2 years for women). The period of observation for calculation of risk of developing cancer in this cohort began on January 1, 1943, at which time the Danish Cancer Registry became operational. Cancers developing in this group of patients were identified by linking their Central Population register number (equivalent to a Social Security number) with the Danish Cancer Registry, which, since its inception in 1943, kept records of all cancers found in Denmark. The authors cautioned that the nonmelanoma skin cancer incidence rates may be under-reported. Incidence rates by sex, by 5-year age groups and by calendar year were applied to appropriate person-years under observation to calculate the number of cancers expected. No skin cancers were reported for any patients and a total of seven patients developed a systemic cancer during the observation period, three of which were lung cancers. However, the number of expected cases for any cancer in this population was 17.3 and the relative risk of cancer was estimated at 0.4 for the entire group. The authors concluded that the use of coal tar in the treatment of atopic dermatitis did not increase the risk of skin or internal cancers. They maintained that, although their conclusion regarding nonmelamoma skin cancer may not be as firm as for internal cancers because the nonmelanoma incidence rates may be under-reported, these results “strongly support the safety of tar treatments in dermatology.”

2.2.2.4 Comparison of Skin Cancer Incidence in Psoriasis Patients to the General Population

The results of studies discussed above that were conducted with psoriasis patients strongly suggest that coal tar therapy does not increase skin cancer incidence in psoriasis patients. However, because psoriasis is a disease of the skin that alters normal skin physiology and cell turnover, it has been suggested that psoriasis patients may be more or less likely to develop skin cancers, when compared with individuals with "normal" or nonpsoriatic skin. In order to clarify this issue, studies have been conducted where the skin cancer incidence in psoriasis patients was compared to the skin cancer incidence in individuals without psoriasis (Alderson and Clarke 1983; Bhate et al. 1993; Halprin et al. 1982; Stern et al. 1984).

Alderson and Clark (1983)

Alderson and Clark (1983) evaluated the risk of skin cancer in a cohort of 8,405 psoriasis patients by comparing the observed cancer incidence in these patients to expected rates for the general population, which were calculated by applying age-, sex-, and calendar-specific cancer rates to person years at risk using data from the Scottish National Cancer Register. The average follow-up period for the psoriasis patients was 11.5 years. Data regarding the various treatments that the psoriasis patients may have received were not available. There were no significant differences in skin cancer risk in the population (observed/expected ratio = 1.17, not significant), in males (O/E ratio =1.26, not significant) or in females (O/E ratio = 1.06, not significant). Based on these results, the authors concluded that there was no evidence that psoriasis was protective or that the treatments that the patients received had carcinogenic effects.

Bhate et al. (1993)

Bhate et al. (1993) conducted a case-control study where skin cancer rates in a cohort of 2,247 psoriasis patients (995 men, 1,252 women) randomly selected from a group of 3,184 patients who had been seen in the Dermatology Department at Royal Victoria Infirmary between 1977 and 1983 were compared to rates observed in 4,494 age- (within 1 year) and sex-matched controls (1,990 men, 2,504 women) without psoriasis. Medical records were examined and the presence or absence of skin and other malignancies, ages at diagnoses, treatments given, and their

duration were noted. All patients found to have both psoriasis and skin cancer and those with skin cancer among the nonpsoriasis population were examined and a history of smoking, occupation, sun exposure, sunburn and skin type was taken. The mean ages of the male and female psoriasis patients were 41 and 41.5 years, respectively. There were no statistically significant differences in skin cancer incidence in the psoriasis patients (males and females combined) or in the male psoriasis patients compared to the control population. However, in female psoriasis patients, the prevalence of skin cancer was marginally increased (p=0.03). In both psoriasis and controls, all skin cancer developed after the age of 30. The mean age at diagnosis of skin cancer in psoriatic men was 58.9 years, compared to 53 years in the controls. In women, the mean ages at diagnosis were 56.6 and 58.2 in the psoriatics and controls, respectively. The ratio of BCC to SCC was comparable between psoriasis patients and controls. The treatment modalities for psoriasis patients with cancer (10 men and 24 women) and without cancer (985 men and 1971 women) were compared. These treatments included coal tar, PUVA, methotrexate, superficial x-ray, dithranol, UV radiation, and topical corticosteroids. The number of patients with each treatment was too small to assess the impact of any one form of treatment. However, since the total treatment load is related to the total duration of psoriasis, an assessment effect of all types of therapy was made from the length of history of the lesion of psoriasis at the time at which the skin cancer was diagnosed, excluding patients less than 30 years of age. The mean duration of treatment for psoriasis in men was 15.6 years in patients with skin cancer and 15.1 years in men without skin cancer. A similar finding was noted in women, with a mean of 16.6 years in women with skin cancer and 17.6 years in women without skin cancer. The authors concluded that they could find no clear evidence of a carcinogenic risk from therapy nor was the increased prevalence of cancers in patients with psoriasis significantly different from control patients without psoriasis.

Halprin et al. (1982)

In contrast to Stern et al. (1985), Halprin et al. (1982) reported an increase in the incidence of skin cancer in psoriasis patients who had not been treated with PUVA. In this study, 150 (147 males and 3 females) psoriasis patients were matched with a randomly selected adult-onset diabetes patient who had been hospitalized for the purpose of diabetes control. Data

regarding year of birth, sex, race, ethnicity, treatment history, smoking history, and dates of first and last hospital visits were collected for each patient and the psoriasis patients were matched with a diabetes patient for year of birth, sex and race. Skin cancer diagnoses were based on pathology reports that included the type and location of skin cancer and the date of diagnosis. Approximately 46% of the psoriasis patients had received more than one form of therapy, including UV light + coal tar, methotrexate, nitrogen mustard, and hydroxyurea, for the condition. There were no statistically significant differences in race, ethnicity or smoking habits between the two groups. However, a significant increase in skin cancer incidence (20 in the psoriasis patients and seven in the diabetics) was reported. The ratio of SCC to BCC was 14:6 in psoriatics and 3:4 in the diabetics. Only one tumor, an SCC on the shoulder, was reported in a non-sun-exposed area in the diabetic patients. The mean age at diagnosis was comparable between the groups, 60 and 62 years in the psoriatics and diabetics, respectively. The authors concluded that skin cancer was more prevalent in the psoriasis group than in the diabetic control group despite the absence of PUVA treatment; however, attribution of this increased risk to a single treatment modality was not possible.

Stern et al. (1985)

A population-based study was conducted by Stern et al. (1985) in order to evaluate the risk of nonmelanoma skin cancer (BCC and SCC) in individuals with and without psoriasis. From a national skin cancer survey a total of 29,757 white persons with nonmelanoma skin cancer from eight different geographic regions, 2,849 cancer patients, aged 20-74 years, were randomly selected based on age and geographic location (SCS group). An additional 7,298 individuals, aged 20-74 years, were selected from the general population and served as controls (GPS group). Persons in each group were interviewed by telephone to identify persons with psoriasis and to assess the prevalence of confounding factors that may be related to both skin cancer and psoriasis. An individual was considered to have psoriasis if they reported having been treated for the condition by a physician. Data regarding the prevalence of confounding factors, including complexion, response to sunlight, and prior exposures to coal tar, ionizing radiation therapy, or sunlamp, were also collected. Skin cancer risk for individuals with a specific characteristic (complexion, therapeutic regimen, etc.) was estimated by multiplying the

proportion of patients with that characteristic by the general risk of skin cancer and dividing by the proportion of the general population with that characteristic. Relative risks were estimated by estimating skin cancer risk for the individuals with a specific characteristic and those without that characteristic and taking the ratio.

Approximately 2.6% of the GPS group, those without skin cancer, and 5.5% of the SCS group, those with skin cancer, had psoriasis. Persons with psoriasis were found to be at a greater, rather than lesser, risk of developing BCC compared to persons without psoriasis. The authors noted that the increase in BCC among psoriasis patients could be associated with either skin type, with persons who have fair complexion at a higher risk, or to previous therapeutic exposures (ionizing radiation, sunlamp, coal tar). This finding could not be attributed to any particular factor (complexion, therapeutic regimen, etc.). The study authors noted that finding may have been the result of surveillance bias because the psoriasis patients were likely under closer dermatologic scrutiny than the general population. The relative risks of SCC were comparable between the two groups. Further, the ratio of BCC to SCC in the psoriasis patients in the SCS group was not significantly different than this ratio in the nonpsoriasis patients in the SCS group. The authors noted that the similar ratio in BCC to SCC in psoriasis patients indicated that psoriasis patients not exposed to PUVA were prone to the same types of tumors as the general population despite their increased exposure to therapies with carcinogenic potential. The authors reported that when compared with the general population, psoriasis patients were likely to develop SCC only after exposures to PUVA and ionizing radiation. The authors concluded that the incidence of nonmelanoma skin cancer was at least as high in psoriasis patients as in the general population.

2.2.2.5 Summary of Epidemiology Studies for Medicinal Use of Coal Tar-Containing Products

Collectively, these data demonstrate the lack of a clear association between the dermal application of pharmaceutical grade coal tar for the treatment of psoriasis or other dermatoses and increases in skin cancer incidence. In a cohort of psoriasis patients who had been treated primarily with coal tar therapy, there was no evidence of increased skin cancer incidence despite an average latent period of at least 20 years (Pittelkow et al. 1981). Moreover, the results of

analyses where other therapeutic regimens were controlled for indicated that long-term PUVA and methotrexate therapy are associated with increases in skin cancer incidence in psoriasis patients; however, there were no associations between coal tar therapy in psoriasis patients in this cohort and an increased incidence of skin cancer (Stern and Laird 1994; Stern et al. 1998). The results of Pittelkow et al. (1981) indicated that skin cancer incidence was not increased in psoriasis patients who had undergone the Goeckerman regimen, which, at the Mayo Clinic, involved the application of an average of 60 grams of coal tar followed by UV-B therapy (Muller and Perry 1984). Further, there were no increases in skin cancer incidence in dermatitis patients who had been treated with coal tar ointments and UV-B light (Jemec and Østerlind 1994; Maughan et al. 1980), with a follow-up period of 38.5 years reported in one study (Jemec and Østerlind 1994). With the exception of the results of Halprin et al. (1982), the results of studies where the skin cancer incidence in psoriasis patients was evaluated provided evidence that psoriasis patients develop skin cancer at about the same frequency as individuals with normal skin (Alderson and Clarke 1983; Bhate et al. 1993; Stern et al. 1984). The increased incidence of SCCs in the skin seen in psoriasis largely reflected increased exposure to known and suspected carcinogens, such as PUVA, cyclosporine, and methotrexate (Stern 1989; Stern and Laird 1994; Stern et al. 1984). These results indicate that the disease processes involved in psoriasis are not protective of skin cancer. Thus, despite the evidence for the potential carcinogenicity of coke oven and industrial grade coal tar from mouse skin painting studies, pharmaceutical preparations containing coal tar have not been shown to cause an increased incidence of skin cancer in patients treated for extended periods of time.

2.2.3 Comparison of Animal Bioassays and Epidemiological Studies

Dermal applications of constituents in coal tar or various coal tar materials are clearly carcinogenic in mice producing tumors in a dose-dependent manner with an incidence rate that reached 100% in some studies (Fraunhofer 1997; Warshawsky et al. 1993). Results from occupational studies are mixed with an increased incidence of skin cancer reported in some studies, while no increases in the incidence of skin cancer were noted in other studies for workers in the same types of occupations. While all the occupational studies have limitations, e.g., poor characterization of exposure, small sample size, the occupational data strongly suggest that

exposure to high, sustained levels of PAHs in CTPs increases the risk of skin cancer when accompanied by exposure to UV light and/or chronic dermal irritation. Consequently, the interaction between PAH exposure, UV irradiation, and health conditions in England prior to the 1940s must be considered (ATSDR 1996).

In order to evaluate the differences in response in humans and mice following dermal exposure to the different CTPs, the incidence of skin cancer in occupational and epidemiology studies in individuals exposed to CTPs and the tumor incidence in mouse studies that used the same materials were compared. Although the chemical composition of different coal tars, pitches, and creosotes that have been evaluated in occupational, clinical, and animal studies varies, studies for comparisons were found for high temperature coke oven tar, coal tar pitch, creosote, and pharmaceutical grade coal tar.

High Temperature Coke Oven Tar

Sherson et al. (1991) evaluated the skin cancer incidence in steel foundry workers exposed to high temperature coke oven tar with exposure durations ranging between less than 10 years to greater than 30 years. There was no increase in the incidence of skin cancer noted in this cohort (65 observed/69 expected = SIR of 0.93). In contrast, the twice weekly topical application of high temperature coke oven tar (at a concentration determined to contain 0.006% BaP) to the shaved backs of C3H mice for 78 weeks resulted in a skin tumor incidence of 39/76 (51%). Nesnow et al. (1982) used coke oven tar in the two-stage initiation/promotion assay. The tumor incidences in male and female mice initiated with the highest dose (10 mg) of coke oven tar were 100% and 90%, respectively.

Coal Tar Pitch

The skin cancer incidence in 4,213 aluminum workers exposed to coal tar pitch volatiles was evaluated by Spinelli et al. (1991). There was no increase in the incidence of skin cancer noted (38 observed/74 expected SIR = 0.51). Hammond et al. (1976) evaluated the mortality due to nonmelanoma skin cancer in 5,939 roofers exposed to coal tar pitch volatiles and dust with BaP concentrations ranging from 14 to 6,000 mg/m^3. Mortality attributed to skin cancer was noted in less than 0.1% of the cohort. A significant increase in mortality due to nonmelanoma

skin cancer was noted (5 cases observed vs. 1.2 cases expected); however, because exposure to UV light is high in this occupation, it is likely that UV light contributed to the increase in mortality. Following the topical application (50 mg/mouse) of coal tar pitch particulates (dust) dissolved in toluene to the shaved backs of C3H/HeJ mice for 80 weeks, the tumor incidence was 12/14 (86%) (Emmett 1986).

Creosote

A study of the skin cancer incidence in 922 workers at wood impregnation facilities in Sweden and Norway was conducted by Karlehagen et al. (1992). The duration of exposure to creosote was reported to be at least 1 year with a follow-up of greater than 20 years. Air concentrations of naphthalene ranged from 0.1 to 11 mg/m^3. A significant increase in nonmelanoma skin cancer was noted (SIR = 2.37) when the incidence rates for plants in Sweden and Norway were combined. The authors stated that since these individuals partly worked outdoors, the contribution from sunlight could not be ignored. In contrast, the incidence of skin cancer was significantly increased in mice dermally exposed twice weekly to 3 mg/mouse creosote and was accompanied by significant increases in ulceration and inflammation of the skin (Fraunhofer 1997).

Pharmaceutical Grade Coal Tar

The incidence of skin cancer in psoriasis patients and patients with other dermal disorders treated with coal tar ointment has been evaluated by several investigators (Alderson and Clarke 1983; Bhate et al. 1993; Jemec and Østerlind 1994; Jones et al. 1985; Maughan et al. 1980; Menter and Cram 1983; Pittelkow et al. 1981; Stern and Laird 1994; Stern et al. 1998). There was no significant increase in the incidence of skin cancer identified in any of the studies. In contrast, 100% of mice developed skin tumors in the two-stage initiation/promotion assay following topical treatment with pharmaceutical grade coal tar (25 mg) followed by promotion with phorbol myristate acetate (Wright et al. 1985) or treated with 1.5% coal tar ointment applied 3-5 times/week for 1 year (total treatments = 186) (Shabad et al. 1970).

As noted above, in contrast to the animal data, no increases in the incidence of skin cancer were reported in psoriasis patients or patients with other dermal conditions treated with

coal tar extensively for more than 20 years. Nor was there a difference in the incidence of skin cancer in psoriasis patients treated with a variety of regimens compared to the general public. As with the occupational studies, these studies have limitations. With the exception of the Pittelkow et al. (1981) study, the amount of coal tar usage or other treatment regimens was either not given or not fully quantified and, in some studies, the cohort size was small (Jemec and Østerlind 1994) or follow-up was short (Grupper and Berretti 1980). However, collectively, the studies described in Section 2.2.1 considered more than 20,000 patients. While collectively this sample size is still too small to have detected a one in 100,000 increase in extra lifetime cancer risk, an increase nonetheless would have been expected if human skin is as sensitive as mouse skin. Patients were exposed to exceedingly high concentrations of coal tar for years. Just during the initial Goeckerman therapy, the average amount of coal tar applied was 3 grams/day for a total of 60 grams during a standard hospital stay. As described in Section 3.0, at-home use increased the total average coal tar usage, as estimated for the Pittelkow et al. (1981) cohort, to an average of 252 grams absorbed. In comparison, the total amount of coal tar applied to the skin of mice in the Fraunhofer (1997) study, of a coal tar mixture with a comparable PAH content to coal tar ointments used medicinally, was 0.47 grams (CTP2, 3 mg/day, 2 days/week, for 78 weeks) for the dose group with a statistically significant increase in skin tumors.

In summary, the incidence of skin cancer in humans resulting from exposure to high temperature coke oven tar, pitch, creosote, or pharmaceutical grade coal tar either is not increased when compared to the expected values or increased only in association with other risk factors such as exposure to sunlight. In contrast, a significant increase in the incidence of skin cancer was consistently observed in mice exposed to high temperature coke oven tar, pitch, creosote, or pharmaceutical grade coal tar, with incidence rates as high as 100% for some mixtures of coal tar. Collectively, these studies suggest that mouse skin develops tumors significantly more readily than human skin.

2.3 Pharmacokinetic Analyses: Species Comparison

One of the considerations in chemical risk assessment using animal data is the evaluation of the pharmacokinetic differences that may exist between the species in which the chemical has been tested and the target human population. Comparative pharmacokinetic analyses are used to

identify the likely active moiety (parent or metabolite) and to determine the concentration of that moiety at the target tissue. Differences in pharmacokinetic activity with regard to absorption, distribution, metabolism, and elimination of the chemical, when quantified using a physiologically based pharmacokinetic model, can provide comparative estimates of target organ or tissue dose that can then be used to extrapolate across species. A fundamental part of the dose-response analysis in a quantitative risk assessment is the determination of the most appropriate dose-metric. Because administered dose or exposure dose do not account for the pharmacokinetic differences that can result in significantly different target tissue doses, administered dose is a poor basis for extrapolating across species. The tumor type of interest in this investigation is nonmelanomatous benign and malignant lesions. Consequently, the target tissue under consideration is the skin, and specifically, keratinocytes in the epidermal layer of the skin (see Section 2.3.1 for a discussion of the relationship of these cells in the skin compartment).

Theoretically, pharmacokinetic analyses can be applied to simple mixtures when the pharmacokinetic behavior of each of the chemicals in the mixture, individually and in combination, is known. However, pharmaceutical grade coal tar is a complex mixture that may contain thousands of compounds, not all of which may be associated with the observed toxicity in the animal model. Moreover, the interactions of these components may enhance (synergism) or inhibit (antagonism) the toxicity of the active constituents in the mixture. When this information is not available, a physiologically based pharmacokinetic analysis of the mixture is not feasible. However, in the case of pharmaceutical grade coal tar, some species differences in pharmacokinetics can be estimated by evaluating the behavior of representative surrogate chemicals present in the mixture.

As discussed in Section 2.1, the experimental evidence strongly implicates PAHs, in particular, metabolites of BaP and other large PAHs, as the active carcinogenic constituents present in coal tar mixtures. In mice, skin painting studies with coal tar mixtures that have been fractionated indicated that more than 95% of the observed carcinogenicity of the mixture in mice can be attributed to the fraction of coal tar-containing PAHs with three or more rings, such as BaP (Grimmer et al. 1984).

PAHs, such as BAP, require metabolic activation in order to form DNA adducts, the first step in tumor initiation. Metabolism by AHH forms the initial epoxide, which is then further

metabolized by epoxide hydrolase to the dihydrodiols (Bickers et al. 1984). The dihydrodiols are further metabolized by AHH to form the proximate carcinogens, the diol-epoxides (Bickers et al. 1984). For BaP, the proximate carcinogen has been identified as BPDE, often referred to as the 7,8-9,10-dihydrodiol epoxide (Bickers 1983). BaP is considered the model substrate for AHH metabolism (Pelkonen and Raunio 1983), and as such, the metabolism of BaP can be used to compare the metabolic capability for other carcinogenic PAHs in different species. Moreover, absorption of BaP through the skin is commonly used as a surrogate for absorption of other large PAHs (Dankovic et al. 1989; Roy et al. 1998). While the carcinogenic potency of a particular coal tar mixture cannot be quantitatively predicted by the concentration of any one individual PAH, such as BaP (Goldstein et al. 1998; Warshawsky et al. 1993), a comparison of the metabolism data on activation of individual PAHs to their carcinogenic metabolites would be indicative of the potential target tissue dose and activity of PAHs in general in the skin compartment of each species.

2.3.1 Comparison of Skin Anatomy and Physiology

The skin is one of the largest organs in mammals and covers an area approximately 20,000 cm^2 in humans (Klein-Szanto et al. 1991; Patrick and Maibach 1994; Rice and Cohen 1996). The skin serves both as a barrier to external insults and aids in the maintenance of homeostasis. Microscopically, as illustrated in Figure 2-1, the skin consists of: 1) the epidermis, which develops from embryonic ectoderm and consists of the outermost layer of epithelial cells, 2) the dermis, which develops from the mesoderm and includes the highly vascular connective tissues that lie below the epidermis, and 3) the adnexal structures, such as hair follicles and glands (Klein-Szanto et al. 1991; Patrick and Maibach 1994; Rice and Cohen 1996). The epidermis and dermis are separated by an undulating basement membrane (Klein-Szanto et al. 1991).

The epidermis primarily consists of stratified squamous epithelium (termed keratinocytes because these cells accumulate keratin protein) that are attached to each other and to the basement membrane by desmosomes and hemidesmosomes, respectively (Rice and Cohen 1996). Melanocytes, which produce melanin in response to UV light, and Langerhans cells, which

function in the immune system, are interspersed throughout the epidermis (Klein-Szanto et al. 1991).

The epidermis is divided into four layers: stratum basal, stratum spinosum, stratum granulosum and stratum corneum (Klein-Szanto et al. 1991; Rice and Cohen 1996). The skin undergoes constant turnover, i.e., the outermost cells are shed and replaced with cells from below with cells progressing through each layer as they mature and differentiate (Rice and Cohen 1996). The cells of the stratum basal contain the stem cells, which are capable of dividing (Klein-Szanto et al. 1991). After cell division, one of the cells detaches from the basement membrane and begins to migrate outward (Rice and Cohen 1996). As the cells become more fully differentiated, they begin to transform morphologically and accumulate keratin proteins. In the stratum spinosum layer, the keratin forms insoluble, intermediate filaments, while in the stratum granulosum, the cells become flattened, are joined by lipid granules and form a protein envelope beneath the plasma membrane. Once the cells reach the stratum corneum, they are no longer viable and consist of approximately 80% keratin; they are eventually shed and replaced (Rice and Cohen 1996). A primary function of the dermis is to provide support to the epidermis (Klein-Szanto et al. 1991; Rice and Cohen 1996). The dermis, which is composed of connective tissue and fibrocytes, blood vessels, nerves, lymphatics and adnexal structures, is separated from deeper tissues by a layer of fat.

A primary function of the skin is to prevent water loss and to serve as a barrier to prevent or limit the penetration into the body of chemicals that come in contact with the skin (Rice and Cohen 1996; Rozman and Klaasen 1996). There is much diversity in the chemical/physical properties of the chemicals to which the skin may potentially be exposed (i.e., water soluble versus lipid soluble; large, complex molecules versus small, simple molecules, etc.). In order to limit the penetration of these molecules, the different layers of the skin have different properties. As discussed above, the skin of mammals consists of multiple layers and for chemicals to enter the dermis where the blood and lymph vessels are located, the chemical must pass through each of these layers. The outermost layer of the skin, the stratum corneum, consists of densely packed keratinized cells that have lost their nuclei, and these cells are not biologically active. Passage though the stratum corneum is usually the rate-limiting process for dermal absorption (Rozman

and Klaasen 1996). Passage through the remaining layers of the skin into the bloodstream is dependent on blood flow, interstitial fluid movement, and interactions with dermal constituents.

Movement into and across the stratum corneum is by passive diffusion (Rice and Cohen 1996; Rozman and Klaasen 1996). Nonpolar, lipophilic substances dissolve into the stratum corneum and diffuse through the lipid matrix between protein filaments, with the rate of diffusion of these types of compounds directly related to lipid solubility and inversely related to molecular weight (Rice and Cohen 1996). However, there are limits to dermal penetration of lipophilic compounds. The majority of the stratum corneum consists of moderately lipophilic components, ceramides, that have lipophilic regions, but also possess some hydrophilic regions. Because of this, for extreme ends of the lipophilic/hydrophilic spectrum, the stratum corneum represents a nearly impenetrable barrier (Rozman and Klaasen 1996). The remaining layers of the skin contain a porous, nonselective, aqueous diffusion medium, which would tend to facilitate the movement of polar compounds, not nonpolar or lipophilic compounds. Diffusion of chemicals through the layers beneath the stratum corneum would also be influenced by interactions with dermal constituents.

Although the skin of mammals is generally similar, there are numerous distinct differences in the normal anatomy and physiology of the skin of mammals, particularly between humans and rodents (Klein-Szanto et al. 1991; Patrick and Maibach 1994). Specific differences include epidermal thickness, number of epidermal layers, cell turnover, hair distribution and the presence of sweat glands in human skin (Table 2.3-1). The epidermis, although variable in thickness in humans, is about 3-fold thicker in humans than in rodents, with mean thicknesses of 100 Fm and 30 Fm, respectively (Klein-Szanto et al. 1991). Moreover, the human epidermis consists of a greater number of layers that are thicker, when compared with rodents.

Cell turnover, the time required for epidermal cells to move from the basal cell layer to the stratum corneum, differs in human skin (Bloom and Fawcett 1975; Epstein and Maibach 1965; Rice and Cohen 1996), when compared with mouse skin (Bertalanffy 1964; Bertalanffy and Lau 1962; Iverson et al. 1968), with cell turnover times generally longer in human skin. Cell turnover times in human skin differ, ranging from 13 to 71 days, depending on the anatomical site, with turnover in the palm requiring approximately 32-36 days and turnover on the anterior surface of the forearm requiring approximately 58 days (Patrick and Maibach 1994). Epidermal

cell turnover in mouse skin ranges from 6 to 34 days (Bloom and Fawcett 1975; Epstein and Maibach 1965; Rice and Cohen 1996). Further, in human skin, the distribution of hair follicles and sweat glands differs considerably (Klein-Szanto et al. 1991).

Other variations can occur with age, sex, and hormonal status (Klein-Szanto et al. 1991). Thus, although the general structure and function of skin are similar in humans and rodents, there are distinct differences in the normal dermal physiology and anatomy between the species. These differences could impact the manner and rate in which a material is absorbed into the skin and the response of the skin to toxic insult (Patrick and Maibach 1994).

2.3.2 Comparison of Percutaneous Absorption

Application of a PAH(s) to either mouse or human skin will result in an initial loading of the chemical(s) to the stratum corneum, where the chemical(s) would tend to remain. Release to the remaining layers of the skin is expected to occur slowly. The anatomic characteristics of the stratum corneum have a significant influence on the penetration of large, highly lipophilic compounds, such as PAHs. Anatomic differences in mouse skin compared to human skin, e.g., the human stratum corneum and supporting epidermis are thicker and comprised of more layers, are expected to result in differences in absorption into and through the stratum corneum to the underlying epidermis containing the target keratinocyte population (Soballe et al. 1996). Differences in absorption will result in a difference in the delivered dose to this target cell population and, consequently, if all else were equal, will result in less chemical(s) available for metabolism to the active constituents (see Sections 2.3.3 and 2.3.4).

Dankovic et al. (1989) considered the absorption of BaP in relationship to other PAHs. The dermal penetration rate of 12 PAHs was assessed by evaluating the rate of disappearance from the site of administration. BaP (25 µg/50 Ull) alone or in combination with coal tar was applied to the shaved backs of female CD-1 mice. The mice were sacrificed at 1, 2, 4, 8, and 24 hours after administration and the applied site was isolated and digested overnight in sodium dodecyl sulfate and Proteinase K. The resident half-life of BaP and 11 other PAHs contained in coal tar ranged from 5.0 h^{-1} (pyrene) to 8.7 h^{-1} (benzo[e]pyrene) with the dermal penetration rate for BaP of 6.7 h^{-1} to be almost the midpoint of that range. BaP was also a reasonable surrogate for absorption of other PAHs in rats (Roy et al. 1987) and guinea pig skin (Van Rooij et al.

1995). Consequently, absorption of BaP was considered a reasonable surrogate to use to assess the comparative absorption of PAHs in mouse and human skin.

Storm et al. (1990) evaluated the absorption and metabolism of BaP in mouse, rat, guinea pig, and human skin. In that study, a layer of skin containing all layers of the epidermis and the upper papillary dermis (minus subcutaneous fat and muscle) was obtained from either female BALB/c or female SENCAR mice at 20-30 weeks of age or from human skin obtained from female surgery patients ranging in age from 37 to 71 years. Radiolabeled BaP [3 µg BaP/cm^2 (11-12 nmol BaP/cm^2)] was applied to a 1.7 cm^2 area of mouse or human skin in a flow-through cell system. Receptor fluid [Eagle's modified minimum essential medium (MEM) with 10% Fetal Bovine Serum (FBS) added to enhance solubility of hydrophobic compounds] was collected in 6-hour fractions for 24 hours. At the end of the 24-hour period, the skin was washed with a commercial detergent to remove unabsorbed material. Both the skin and receptor fluid were analyzed for parent and metabolites. With the BALB/c and SENCAR mouse, approximately 60% of the applied dose was absorbed, while approximately 30% of the applied BaP was absorbed by human skin. In both species, the majority of the absorbed radioactivity remained in the skin compartment. Less than 5% of the amount applied was identified in the receptor fluid collected for both mouse and human skin.

In a subsequent study conducted by the same group (Moody et al. 1995), radiolabeled BaP was again applied to human skin and the percent absorption was determined using the same protocol described for Storm et al. (1990). The average absorption of BaP into and through the skin compartment was reported to be 33% of the initial dose applied (average = 9.1 µg/cm^2). In a separate percutaneous absorption study conducted by Wester et al. (1990), radiolabeled BaP in acetone was applied to human skin and the amount of absorption was determined using the same protocol described for Storm et al. (1990) and Moody et al. (1995), with the exception that the receptor fluid was human plasma. Approximately 25% of the amount applied (dose not specified) was absorbed into and through the skin over a 24-hour period. The values for absorption in human skin reported by Moody et al. (1995) and Wester et al. (1990) are in agreement with the value of 30% absorption reported in the previously described study conducted by Storm et al. (1990).

In summary, absorption of BaP was considered to be an appropriate surrogate to use to assess the comparative absorption of PAHs in mouse and human skin. Absorption of BaP in mouse skin *in vitro* was estimated to be 60% of the amount applied. In contrast, absorption of BaP in human skin *in vitro* was estimated to be 30%, which is half that observed for mouse skin. This rate of BaP absorption in human skin was confirmed in two subsequent experiments. Thus, the amount of BaP absorbed into the skin compartment and available for metabolism in human skin would be expected to be half that expected for mouse skin.

2.3.3 Comparison of Enzyme Activity

Due to similarities in structure between the PAHs, there are similarities in their metabolism (ATSDR 1995). A model for PAH metabolism is BaP, which has been extensively studied (ATSDR 1995). AHH is the first enzyme in the metabolic transformation of PAHs to the active metabolic species, the diol-epoxides (Bickers 1983; Hukkelhoven et al. 1982). The metabolic activity of AHH is measured by the rate of the formation of a BaP metabolite, 3-OH-BaP, expressed in pmoles 3-OH-BaP/min/mg protein. Basal activity of AHH has been measured in mouse skin (Das et al. 1986a; Kinoshita and Gelboin 1972; Sato and Tomita 1998; Storm et al. 1990; Thompson and Slaga 1976) and in human skin (Alvares et al. 1972; Bickers et al. 1984; Bickers and Kappas 1978; Das et al. 1986a; Levin et al. 1972; Storm et al. 1990) (Table 2.3-2). As importantly, AHH has been shown to be inducible by PAHs in mouse skin (Das et al. 1986a; Kinoshita and Gelboin 1972; Thompson and Slaga 1976) and in human skin (Bickers and Kappas 1978; Das et al. 1986a; Levin et al. 1972).

AHH induction was reported by Thompson and Slaga (1976) in mouse epidermal cell homogenates incubated with 100 nmoles BaP and to which BA was added. After 30 minutes incubation, AHH activity increased from approximately 1.1 (3-OH-BaP)/min/mg protein to 8.5 (3-OH-BaP)/min/mg protein for an 8-fold induction. Similar induction was noted when 100 nmoles of DMBA or BA were applied directly to the shaved dorsal skin of Swiss mice and the epidermis harvested at times 0 up to 24 hours post-application (Kinoshita and Gelboin 1972). When BaP (100 nmoles) was added to the epidermal homogenate, peak induction was noted following 8 hours of application of DMBA or BA with 3-OH-BaP levels increased from 4-fold (DMBA) to 8-fold (BA). Topical application of 200 nmoles DMBA, 3-MC, 1,2,5,6-

dibenzanthracene, 1,2,3,4-dibenzanthracene, or BA applied to shaved mouse skin and the epidermal layer harvested within 12 or 24 hours following application resulted in AHH induction for these compounds that ranged from 4- to 12-fold (Thompson and Slaga 1976).

The potential tumor promotional ability of a single PAH may not be adequately assessed based on its AHH induction alone compared to other PAHs (Bickers 1983; Thompson and Slaga 1976). Other enzymes, such as epoxide hydrolase or glutathione transferase, are involved in the biological activation of a PAH (Bickers 1983). However, the relative AHH induction potential for any single PAH across strains or species should be predictive of the comparative metabolism of that compound or, in the case of coal tar, a group of compounds.

The correlation between AHH induction and tumor promotion has been evaluated in AHH-inducible mice (BALB/c and C57BL/6) and AHH-noninducible mice, DBA/2 in a two-stage skin tumor promotion study (Sato and Tomita 1998). AHH-induction in this study was evaluated using the frequency of DNA-damaged cells as the metric. Twelve hours following administration of DMBA (100 mg/kg, intraperitoneally), the frequency of DNA-damaged cells increased 8- to 10-fold in the two strains of AHH-inducible mice but about 2- to 3-fold in the AHH-noninducible mice. Skin tumors were noted in 50-60% of the AHH-inducible mice and 20% of the AHH noninducible mice following DMBA-initiated (100 µg), croton oil-promoted skin. Further, inhibition of AHH activity by 7,8-benzoflavone resulted in a 70% reduction in the number of DMBA-initiated (20 µg), croton-oil induced skin tumors in Swiss mice (Kinoshita and Gelboin 1972).

The relative AHH activity in mouse and human skin for the same PAH has been assessed. Storm et al. (1990) compared the basal AHH levels in mouse and human epidermal skin sections contained in flow through cell systems to which 3 µg BaP/cm^2 was applied for 30 minutes. Basal AHH levels differed by a factor of approximately 10, with the basal activity in SENCAR mouse skin and human skin of 3.35 and 0.24 pmol 3-OH BaP/min/mg protein, respectively.

AHH activity in human neonatal foreskin or adult epidermal homogenates incubated with BA (10-13 µM) for 16-24 hours increased approximately 3-fold from 0.1 to 0.33 pmole 3-OH BaP/min/mg protein (Alvares et al. 1972; Levin et al. 1972) and from 0.06 to 0.165 pmole 3-OH

BaP/min/mg protein (Bickers et al. 1984) for the foreskin and epidermal homogenates, respectively.

AHH induction was assessed 24 hours following topical application of a 20% coal tar solution to the skin of normal volunteers (Bickers and Kappas 1978). An approximate 3- to 4-fold induction of AHH activity in biopsy homogenates was noted compared to unexposed skin in the same individuals (from 0.045-0.165 pmole 3-OH BaP/min/mg protein).

Comparative induction of AHH activity following application of crude coal tar has been measured in a human xenograft study (Das et al. 1986a). Twenty-four hours after topical application of 0.1 ml of crude coal tar to the skin of either the human xenograft or on the distal skin of the host nude mice (BALB/C), AHH levels in the mouse epidermal layer AHH induction were approximately 8 times higher (16.45 3-OH-BaP/min/mg protein) compared to human skin (2.29 3-OH-BaP/min/mg protein).

In summary, basal levels of AHH in mouse and human skin differ by a factor of approximately 10. AHH activity in both mouse and human skin is inducible with PAHs and coal tar but the magnitude of that inducibility is considerably greater in mouse skin than in human skin.

When induction of AHH by BA was compared between mouse and human skin tested by similar protocols, induced AHH activity (in pmole 3-OH BaP/min/mg protein) in mouse skin was 40-75 times greater than in human skin (Alvares et al. 1972; Kinoshita and Gelboin 1972; Levin et al. 1972; Thompson and Slaga 1976) (Table 2.3-2). Induction with coal tar was 8-10 times greater in mouse skin than in human skin (Bickers and Kappas 1978; Das et al. 1986a) (Table 2.3-2). While AHH induction is one of the enzymes in the cascade of biological activation of PAHs in human and mouse skin, differences in basal and induced levels of AHH activity reflect a difference in the potential of human skin to metabolize constituents in coal tar to the active metabolites, as discussed in the next section.

2.3.4 Comparison of Metabolism

The skin's viable epidermal layer below the outer stratum corneum is metabolically active and imparts to the skin a wide range of biotransforming capacity (Bickers 1983; Pannatier et al. 1978). Many compounds that penetrate the stratum corneum intact may be biotransformed prior

to entering the systemic circulation. The amount of applied dose that ultimately reaches the systemic circulation is a function of both the rate of absorption of the chemical into the skin compartment and the relative activity of metabolizing enzymes (Storm et al. 1990).

Both human skin and mouse skin are capable of metabolizing BaP to diol metabolites; however, the extent of that metabolism differs significantly between the two species. Differences in metabolism have been evaluated *in vitro* in whole skin sections and epidermal keratinocytes (Berry et al. 1977; Das et al. 1986b; Hall and Grover 1988; Modly et al. 1986; Storm et al. 1990).

Differences in absorption and metabolism were evaluated in several laboratory species and in human skin (Storm et al. 1990). Radiolabeled BaP (3µg/cm^2 equating to 12 nmoles/cm^2) was applied to 1.7 cm^2 of viable mouse, rat, guinea pig, or human skin held in flow-through cells. After 24 hours, the total amount of radiolabel in the skin compartment and receiving fluid (10% FBS) was measured. Total BaP metabolites and specific metabolic fractions, e.g., total diols, were measured. Of the amount applied (3 µg BaP cm^2), 6% of the applied amount (0.7 nmol total BaP metabolites/cm^2 skin) was metabolized over the 24-hour exposure duration in mouse skin. In contrast, metabolism of BaP in viable human skin to which BaP (3 µg BaP/cm^2) was applied, was not detected above that in the 10% FBS controls. The limit of detection of the assay was 6 pmol BaP/cm^2 skin. If it is assumed that BaP metabolites were present in human skin at the detection limit, then the maximum percentage of the applied amount that would have been metabolized was 0.05%. Thus, mouse skin produced at least 120 times more total BaP metabolites than human skin.

Further, the conversion of BaP to the BaP diols was greater in mouse skin than in human skin. In the Storm et al. (1990) study, the total BaP metabolites produced in 24 hours following application of 3 µg BaP/cm2 to mouse or human skin was separated into the relevant metabolic fractions. Data were reported only for the mouse skin; however, of the total amount of metabolites formed, 0.7 nmoles/cm^2, approximately 0.18 nmoles/cm^2, was identified as BaP diol metabolites. This represents 1.5% of the amount to mouse skin. Production of total BaP diol metabolites in human skin was evaluated by Hall and Grover (1988). In a study by Hall and Grover (1988), normal human skin trimmed of excess fat and connective tissue was maintained in tissue culture in Eagle's minimal essential medium (MEM) supplemented with fetal calf serum (FCS). Approximately 25 µg radiolabeled BaP/cm^2 (equivalent to 100 nmoles/cm^2) was applied

to viable normal human skin samples of varying sizes (total amount applied ranged from 150 to 600 µg BaP). After 17-20 hours of exposure, both the skin sample and receptor fluid were evaluated for the presence of metabolites, in particular the diol metabolites. Total diol BaP metabolites recovered was approximately 15 pmoles/cm^2 skin. Based on the amount applied, 0.015% of the applied amount of BaP was metabolized to the diol metabolites recovered from both the skin compartment and the receptor fluid.

Of the BaP diols produced, the 7,8-BaP-diol is the key precursor to the production of the proximate carcinogenic metabolite, BPDE (Bickers 1983). The production of the 7,8-BaP-diol in mouse and human skin was compared. According to Hall and Grover (1988), only 42% of the total BaP diol metabolites formed in human skin following application of 100 nmoles BaP/cm^2 to human skin in tissue culture for up to 72 hours were 7,8-BaP-diol. In contrast, an average of 70% of the total BaP diol metabolites produced in epidermal tissue from CD-1 and SENCAR mice following dermal application of BaP was identified as the 7,8-BaP-diol (Berry et al. 1977; DiGiovanni 1989).

In the experiment conducted by Berry et al. (1977) whole CD-1 mouse skin homogenate was incubated with 0.2 µmole BaP for 60 minutes. Of the total diol metabolites formed, 75% were identified as 7, 8-BaP-diol. Approximately 65% of the total diol metabolites formed in whole skin homogenates from SENCAR mice was identified as 7,8-BaP-diol [percent metabolism was estimated using digital analysis of the HPLC chomatogram in DiGiovanni (1989); protocols used in the experiment were presented in a previous report]. Using these data, a comparison of the total amount of BaP that is applied to either human or mouse skin in tissue culture that is metabolized to the 7,8-BaP-diol can be estimated as follows. The percentage of the applied amount that is metabolized to the BaP diol, which was 1.5% in mice (Storm et al. 1990) and 0.015% in human skin (Hall and Grover 1988) is multiplied by the percentage of the total diol metabolites that was identified to be the 7,8-BaP-diol, which was 70% in mice (Berry et al. 1977; DiGiovanni 1989) and 42% in human skin (Hall and Grover 1988). The percentage of the applied amount of BaP that was estimated to be converted to the 7,8-BaP-diol was 1.05% in mice and 0.0063% in human skin. Therefore, the production of 7,8-BaP-diol, the precursor to the BPDE active metabolite, is more than 160 times greater in mouse skin than in human skin.

Similar differences in metabolite formation have been obtained in cultured mouse and human keratinocytes. Mouse keratinocytes cultured in the presence of 1 µg/ml BaP for 20 hours produced approximately 60 pmol BaP-7, 8-diol/mg protein/24 hours and 180 pmol 3-OH BaP/mg protein/24 hours (Das et al. 1986b). In contrast, human keratinocytes cultured in similar conditions produced 1.1 pmol/mg protein BaP-7, 8-diol and 0.8 pmol/mg protein 3-OH BaP (Modly et al. 1986). The percentage of all BaP diol metabolites identified as 7,8-BaP-diol in mouse keratinocyte homogenates was approximately 60% (Das et al. 1986b). Thirty-eight percent of all BaP diol metabolites were identified as 7,8-BaP-diol in human keratinocyte homogenates (Modly et al. 1986).

Comparative metabolism of chrysene in mouse, rat, and human skin has been evaluated (Weston et al. 1985). Dorsal skin from either Wistar rats (a resistant strain) or Swiss mice (a sensitive strain) and normal human skin (removed at surgery) were maintained in tissue culture and treated with a dermally applied dose of radiolabeled chrysene (0.15 µmole/cm^2) for 18 hours. Both the amount of dihydrodiols produced and the type of dihydrodiol differed between mouse and human skin. Mouse skin produced up to 6 times more total dihydrodiols than human skin, with the chrysene-5,6-diol the predominant metabolite. The predominant metabolite in rat and human skin was the chrysene-3,4-diol. Moreover, the conversion of chrysene to the trio-epoxide was identified in rodent skin but not in human skin. The authors suggested that the presence of the OH-group substitution of chrysene-diol-epoxides indicated that the presence of the OH-group in the 9-position would be expected to enhance the reactivity of the “bay-region” diol epoxide.

In summary, these studies illustrated three differences between mouse and human skin: 1) mouse skin produced more total BaP metabolites than did human skin (Storm et al.1990); 2) mouse skin produced more diol metabolites than did human skin (Hall and Grover 1988; Storm et al. 1990); and 3) mouse skin produced more of the diol metabolite, 7,8-BaP-diol, which is the precursor to the active metabolite, BPDE, than did human skin (Berry et al.1977; Das et al. 1986b; DiGiovanni 1989; Hall and Grover 1988; Modly et al. 1986).

2.3.5 Summary of Pharmacokinetic Comparisons

A comparison of the metabolic capability of mouse and human skin was conducted. Recent studies suggest that the differences in response to topical exposure of coal tar between human skin and mouse skin may be explained in part by differences in absorption and metabolism of BaP and other PAHs to the active metabolites, as well as subsequent biological consequences of metabolite formation. Collectively, the studies suggest that while the metabolic capability of mouse and human skin is qualitatively similar, significant quantitative differences in metabolism are evident. Conversion of constituents in coal tar to the potentially active metabolites, such as the BPDE, is significantly different in human skin compared to mouse skin. Formation of active metabolites in human skin may be as much as 100-fold less than in mouse skin. Human skin has also been shown to possess less AHH, one of the key enzymes responsible for metabolic activation of PAHs. Consequently, it is expected that at low exposure concentrations, target tissue (epidermal skin compartment) doses of known carcinogenic metabolites would be less in human skin compared to mouse skin.

2.4 Sensitivity to Tumor Promotion: Species Comparison

The multistage mode of action of PAHs in the production of SCCs in the mouse has been extensively studied and well characterized (Boutwell 1978; O'Brien 1976; Yuspa 1994). This multistage model requires an interaction of the active metabolite with intracellular target molecule, usually DNA, promotion to the premalignant state, and progression to a tumor. A complex mixture of PAHs, such as that found in coal tar, contains those PAHs that are thought to be complete carcinogens, such as DMBA or BaP, but also other constituents that have the ability to promote the initiating activity of DMBA or BaP. Whether acting in tandem, i.e., an initiating dose of the active metabolite of a potentially carcinogenic PAH contained in a coal tar mixture along with other constituents that promote and transform the initiated cell to malignancy, or whether the active metabolite is present at high enough doses to be both the initiator and promoter of these lesions, the mode of action for that promotion in murine skin is the same. Both carcinogenic PAHs, such as DMBA or BaP, act through the same biochemical cascade for classic promoters, such as TPA (Boutwell 1978). As described in the following sections, with PAHs,

the induction of ODC is likely an obligatory, precursor event in mouse skin carcinogenesis (O'Brien 1976).

2.4.1 Evidence for Differences in Sensitivity to Tumor Promotion with PAHs

2.4.1.1 *In Vitro* Cell Transformation Studies

In vitro studies suggest that human epithelial cells may be more resistant than mouse cells to carcinogenic effects induced by PAHs. Kuroki et al. (1989) cultured human epidermal cells in the presence of BaP (5-15 µM) and the tumor promoter TPA (1-100 ng/ml). After 110 or 170 days in culture following exposure to BaP and promotion with TPA, no evidence of neoplastic transformation was observed. In an experiment conducted by Fox et al. (1975), human epithelial cells were cultured for 6-8 days in the presence of BaP (39 µM) with or without an enzyme-inducing mixture [consisting of BaP, methylcholanthrene (MC), DMBA, pyrene, and pyrenequinone]. Although the full spectrum of BaP metabolites was present in the culture medium, the authors reported no neoplastic transformation occurring in human epidermal cells. Kuroki et al. (1989) suggested that the lack of *in vitro* transformation of human cells may be related to their chromosomal stability.

In contrast, Syrian hamster embryo (SHE) cells (1.98-7.92 µM BaP, duration 7days) or two strains of mouse cell lines, BALB/3T3 (0.99-3.96 µM BaP, duration 72 hours) and C3H (9.9 µM BaP, duration 24 hours), were shown to readily undergo neoplastic transformation when cultured in the presence of BaP (Sala et al. 1987) with no promotion. Interestingly, the cells derived from the C3H mice, the same strain used in the study conducted by Warshawsky et al. (1993), were highly sensitive to the carcinogenic effects of BaP, with transformed colonies being formed following only a 24-hour exposure. Hence, mouse cell lines underwent neoplastic transformation in less time (24-72 hours) and at lower doses (0.99-9.9 µM BaP) in the absence of a promoter than human cell lines cultured with BaP at concentrations up to 39 µM BaP for 6-8 days in the presence of a classic promoter, TPA.

In vitro transformation of human epithelial cells has been reported for other agents in human epidermal keratinocytes (HEKs) immortalized by adenovirus12 and simian virus 40 (Ad12-SV40) (Banks-Schlegel and Howley 1983; Rhim et al. 1986) or in human foreskin epithelial cells in which the transition from G_1 to S phase had been intentionally accelerated

(Milo et al. 1981). In these systems, human epithelial cells were transformed by chemicals, such as N-methyl-N'-nitro-nitrosoguanidine (MNNG) or by UV-B irradiation. When considered in the context of initiation/promotion, these data are consistent. Sarcoma virus induced ODC activity in chick embryo cells (O'Brien 1976). As will be discussed in detail in Section 2.4.2, ODC activation was necessary for the promotion of initiated cells (Boutwell 1978; O'Brien 1976). The possibility exists that the process by which virus is produced in an immortalized cell line may be mediated by ODC activation. In combination with an initiating dose of UV-B or MNNG, human cells may be transformed.

2.4.1.2 *In Situ* Human Xenograft Studies

Human xenograft studies provide a useful model to study human skin carcinogenesis (Atillasoy et al. 1997; Soballe et al. 1996). Xenograft human skin maintains its characteristic histologic architecture and immunophenotype throughout the lifespan of the host (Atillasoy et al. 1997) and retained metabolic competence (Das et al. 1986a). *In situ* studies in which human skin xenografts were exposed by direct topical application to PAHs provided evidence that human skin is substantially less sensitive than mouse skin to the dermal carcinogenic effects of PAHs. Administration of carcinogenic PAHs at doses that caused tumors in mice failed to produce tumors in human skin grafted to NC nude mice or SCID mice (Graem 1986, Urano et al.1995). The conclusions reached in both studies were consistent. These results indicate that susceptibility of human skin to these carcinogenic stimuli is much lower than that of mouse skin (Urano et al. 1995).

In the study conducted by Graem (1986), viable Caucasian human skin grafts (from the anterior aspects of the thigh) from four adult donors were transplanted to the dorsal region of male NC nude mice. Following transplantation, the mice were then divided into eight treatment groups (with 5-18 mice/group). The response to DMBA (1 mg/50 µl acetone/application), an initiator, with and without pretreatment or promotion post-treated was evaluated. Pretreatments consisted of two weekly applications of acetone (50 µl/application) or TPA (10 µg/application) for 5 weeks, or a single dose of UV-B irradiation (0.1 J/cm^2) 7 days prior to the initiation phase. The initiation treatments consisted of two applications of either acetone for controls (50 µl/application) or DMBA at 3-day intervals. Promotion treatments were two weekly applications

of TPA (10 µg/application) or control (no treatment) for the duration of the experiment. All treatments were applied to the human skin xenografts. The application sites were then observed for up to 32 weeks after the initial treatment. Papillomas and SCCs were noted at the well-defined graft margin between the mouse skin and the human skin graft in 35-50% of the mice in all groups treated with DMBA and followed by TPA. Pretreatment with TPA or UV-B did not increase the tumor incidence rate. In 20 of the 22 animals with tumors, tumors emerged at the graft margins within 8 weeks of application of DMBA. However, no tumors were observed in the centers of the DMBA-treated human skin grafts where the test article was applied within the 32-week observation period. After labeling with two markers specific for mouse skin, but not present in human skin, microscopic examination determined that all of the tumors found at the graft margin originated from mouse epithelial cells. Neither pretreatment with TPA or UV-B irradiation, nor post-treatment with TPA resulted in the production of tumors in human skin to which the DMBA or TPA were directly applied.

Xenografts from fetal skin were similarly treated to evaluate potential age-dependent differences in sensitivity (Graem 1986). As with xenografts from adult skin, application of DMBA followed by TPA promotion readily produced papillomas and SCCs of mouse epithelial cell origin at the margins of the graft, but not in the human fetal xenograft. Further, epidermal tumors developed in BALB/c xenografts transplanted to male NC nude mice following application of DMBA, indicating that the grafting process itself did not interfere with tumor production.

In another human skin graft study conducted by Urano et al. (1995), 200-300 nmol DMBA, 200 nmol BaP, 1.5 µmol MC, or 1.5-3 µmol MNNG was applied topically, beginning 2 months after transplant, once a week to human skin (from breast, scalp, or pubic area) grafted to SCID mice. Topical application was continued weekly until mice showed a wasting syndrome or died (25-30 weeks). The first three chemicals, DMBA, BaP, and MC, require metabolic activation by AHH, whereas, MNNG requires no metabolic activation. Each treatment group had a corresponding control group (dorsal skins of SCID mice) that received the same treatments as the human xenografts on other SCID mice. All treatments failed to produce skin tumors in human skin grafts where the material was applied, but did result in epithelial tumors of mouse

origin at the graft margins in 100% of the mice in each group. Further, all of the treatments induced skin papillomas in 100% of the control SCID.

As a check on the integrity of the transplanting process, CD-1 mouse skin was grafted onto the dorsal side of SCID mice and subjected to the same treatment regimens as human skin xenografts (Urano et al. 1995). Tumors were noted in the allogenic CD-1 mouse skin grafts transplanted to SCID mice following treatment with 200 nmol DMBA, indicating that the lack of tumorgenicity in human skin grafts was not a result of the grafting process.

Induction of tumors in human skin xenografts with a combination of DMBA initiation and UV-B initiation/promotion have been investigated (Atillasoy et al. 1997; Soballe et al. 1996; Urano et al. 1995). In the study by Urano et al. (1995), human xenografts on SCID mice received a combination of DMBA (100 nmol/week) and UV-B irradiation (480 J/m^2 /day, 5 days/week). After 25 weeks of observation, no preneoplastic lesions or tumors developed in the human skin; however, papillomas and SCCs of mouse epithelial origin developed at the graft margins by the tenth week.

In contrast, the induction of epithelial tumors in human xenografts using neonatal or adult foreskin following DMBA and UV-B treatment has been demonstrated (Atillasoy et al. 1997; Soballe et al. 1996). In the study conducted by Soballe et al. (1996), human neonatal foreskin or adult skin was grafted onto the dorsal side of CG17 SCID mice. Four to 12 weeks after application of the human skin grafts, groups of grafted mice were assigned to treatment groups that received no treatment, treatment with UV-B (500 J/m^2) three times weekly, or a single topical application of 4 µg of DMBA in acetone. Animals treated with DMBA were further divided and received either no further treatment, promotion with UV-B light three times weekly, or promotion with both UV-B and topical application of 50 µl of 0.2 µm TPA three times/week. Treatments continued for an average of 10 months with median survival for all groups of 11 months. No changes were noted in untreated or DMBA-initiated, unpromoted human skin grafts. In contrast, actinic sites, indicative of human squamous hyperproliferation, were found in all chronic UV-B-exposed human skin grafts by 4 months of treatment or in DMBA-initiated and UV-B- or UV-B-TPA promoted human skin grafts by 9 months. SCCs were found in only two (3.6%) of the DMBA-initiated, UV-B-promoted human skin grafts with the first tumor noted at 6 months. SCCs were not found in the skin grafts treated only with UV-B or in the DMBA-

initiated, UV-B and TPA-promoted group. Murine tumors developed at the skin graft margins in all treated groups beginning 3 months after the beginning of treatment and were seen in 100% of the UV-B alone group, 31% of the DMBA alone group, and 100% of the DMBA-UV-B-TPA group.

In the Atillasoy et al. (1997) study, human neonatal foreskin was transplanted to the dorsal side of recombinase activating gene-1-deficient (RAG-1) mice. Treatment began 4-6 weeks after transplant and consisted of an untreated control group and three treated groups: DMBA alone (a single application of 4 µg in 50 µl acetone), UV-B treatment only (500 J/m^2, three times/week), or a combination of DMBA and UV-B. The median observation period was 10 months, with a range of 3 to 16 months in all groups. At the end of the observation period (16 months), DMBA alone did not produce an increase in benign lesions (defined milia and epidermal cysts), actinic keratosis, or SCCs in the human skin xenograft. In contrast, UV-B treatment alone resulted in benign lesions in 43% of the human xenografts, with actinic keratoses developing in 10% of the xenografts within 4 months of the initiation of treatment. Slightly increased incidence rates, compared to the incidence rate observed with UV-B alone, were seen with the combination of DMBA and UV-B treatment (54% with benign lesions and 19% with actinic keratoses). Approximately 10% of the UV-B treated human skin developed SCCs within 9 months with the first tumor developing by 4 months. This mouse model allowed for a longer observation period such that benign and malignant lesions in human skin were produced in response to UV-B alone but not DMBA alone.

There are three possible differences in the responses seen with UV-B irradiation in human skin. In the Urano et al. (1995) study, adult autopsy tissue from individuals who ranged in age from 61 to 83 years old was used, while in the Soballe et al. (1996) and Atillasoy et al. (1997) studies, neonatal foreskin tissue was used as the xenograft. The possibility exists that neonatal skin, which has fewer melanocytes, may be more sensitive to induction by UV light than adult skin. Secondly, the length of observation was longer in the Soballe et al. (1996) and Atillasoy et al. (1997) studies. Lastly, Soballe et al. (1996) determined that application of 500 J/m^2/day for up to 3 days/week to be the maximum repeated dose tolerated without clinical evidence of burning.

In summary, UV-B exposure produced benign lesions in human xenografts within 4 months of treatment (Atillasoy et al. 1997; Soballe et al. 1996). In contrast, DMBA administered weekly for 6 months (Urano et al. 1995) or as a single treatment followed by chronic administration of the tumor promoter, TPA, for 8 to 16 months (Graem 1986; Soballe et al. 1996) did not result in the production of any benign or malignant tumors in the human skin xenografts. SCCs did develop in human skin xenografts when those skin grafts were exposed to DMBA followed by UV-B promotion within 9 months of treatment. Several conclusions can be reached.

- The human xenograft model is capable of demonstrating the potential carcinogenicity of dermally applied agents. Human skin tumors were produced in response to UV-B light alone within the time frame that did not result in the production of human skin tumors when DMBA was applied either alone or with TPA promotion. Application of DMBA alone was insufficient without additional UV-B promotion to produce tumors in human skin (Atillasoy et al. 1997; Soballe et al. 1996).

- UV-B-promotion, but not TPA-promotion, resulted in the production of SCCs in human xenografts initiated with DMBA. There is no evidence that TPA is a tumor promoter in human skin (Chida and Kuroki 1984). Further, as discussed in the next section, the biochemical cascade of tumor promotion in mouse skin may be mediated by a prostaglandin-dependent pathway that may not be operative in human skin. It is possible that the initiation-promotion mechanism for carcinogenic PAHs, either as a single agent, such as DMBA or BaP, or coupled with tumor promoters, such as TPA tumor promotion that easily results in skin tumors in mice, is unlikely to produce skin tumors in humans. In contrast, the promotional trigger in human skin may be UV-B light, which also appears to have a promotional mechanism in human skin that differs from that in mouse skin. UV-B promotion of PAH-initiated cells would be consistent with the mode of action for the production of skin cancers noted in occupationally-exposed workers.

- Up to 100% of NC and SCID mice developed malignant skin tumors at the graft margins in all studies and with all treatment modalities. In contrast, only 3.6% (Soballe et al. 1996) and 10% (Atillasoy et al. 1997) of human skin xenografts developed malignant skin tumors and only with DMBA and UV-B treatments. It was the conclusion of several authors that the use of the mouse skin model may significantly overestimate the human carcinogenic potential of tested agents (Atillasoy et al. 1997; Soballe et al. 1996; Urano et al. 1995).

- There is an additional interesting observation from these studies. Skin tumors developed at the graft margins in NC mice (Graem 1986) and SCID mice (Soballe et al. 1996; Urano et al. 1995). Skin tumors did not develop in the RAG-1 mice used in the study conducted by Atillasoy et al. (1997). According to Atillasoy et al. (1997), the RAG-1 mice do not have a genetic defect in DNA repair and their darkly pigmented skin appeared to be more protective from UV-induced damage. This suggests that mice routinely used to assess the carcinogenicity of dermally applied PAHs may be more sensitive to these chemicals because of their DNA repair capabilities.

2.4.2 Consideration of the Basis for Species Differences in Tumor Promotion with PAHs

2.4.2.1 Comparison of DNA Adduct Formation and DNA Repair

The experimental evidence indicates that exposures to PAHs and to coal tar-containing products result in the formation of DNA adducts in both mouse and human skin (Phillips et al. 1990; Schoket et al. 1988a, 1988b). Although the magnitude of DNA adduct formation is not predictive of the potency of the PAH (Goldstein et al. 1998; Harris 1987; van Schooten and Godschalk 1996), the differences in the formation of PAH-DNA adducts in mouse skin and in human skin, and differences in the repair of these adducts in the two species should be considered when evaluating the intraspecies and interspecies differences in susceptibility to carcinogenic PAHs.

PAH-DNA adducts have been shown to form *in vivo* in both mice and humans following exposure to PAH-containing mixtures such as coal tar (Goldstein et al. 1998; Phillips et al. 1990). PAH-DNA adducts have been shown to form *in vivo* in both mice and humans following

exposure to PAH-containing mixtures such as coal tar ointment (Phillips et al. 1990; Schoket et al. 1990); however, the formation of DNA adducts in human skin appears to be less when compared to mouse skin (Phillips et al. 1990). PAH-DNA adduct levels were determined in punch biopsies taken from psoriasis patients treated for five days with coal tar ointment in a hospital setting (Phillips et al. 1990; Schoket et al. 1990). The biopsies were collected 24 hours after the final treatment with coal tar ointment. The mean level of adducts in human skin punch biopsies was 0.22 fmol/µg DNA. Mouse skin was treated experimentally with 45 mg of 1.5% coal tar ointment for 5 days. The animals were sacrificed at 24 hours after the last treatment, the treated area was excised, and the level of DNA adduct determined (Schoket et al. 1990). The mean level of DNA adducts in mouse skin following treatment with coal tar ointment was 0.50 fmol.µg DNA, over 2-fold greater than the level of DNA adducts formed in human skin (Phillips et al. 1990). It can be assumed that the investigators designed the mouse experiment in an attempt to mimic the clinical setting and treatment of psoriasis patients, and, therefore, it was likely that doses of coal tar ointment were comparable. These data suggest that human skin forms fewer PAH-DNA adducts than does mouse skin when treated with coal tar ointment.

Phillips et al. (1990) reported that the level of PAH-DNA adducts appeared to be relatively constant regardless of the PAH mixture used. In this study, the level of PAH-DNA adducts differed by only one order of magnitude (adduct levels ranged from 0.05 to 0.5 fmol/Fg DNA) in mouse skin exposed *in vivo* to several PAH-containing mixtures, including pharmaceutical grade coal tar (20% solution, coal tar ointment, creosote, juniper tar, and used motor oil). Explants of normal human skin exposed to bitumen, creosote, crude coal tar, coal tar ointment, and juniper tar at the same doses used in the mouse skin, resulted in PAH-DNA adducts ranging from 0.19 to 0.54 fmol/µg DNA (Phillips et al. 1990). Although PAH-DNA adduct levels differed only approximately 2-fold in mouse and human skin treated *in vivo* with coal tar ointment (0.5 fmol/Fg DNA in mouse skin compared to 0.22 fmol/Fg DNA in human skin), the complexity (i.e., number of different adducts) of adduct formation was less in human skin (Phillips et al. 1990). Additionally, the autoradiographs of digest of DNA from mouse and human skin exposed to used lubricating oils clearly showed a qualitative difference in the PAH-DNA profiles between mouse and human skin, with human skin being less complex, i.e., the number of specific PAH-DNA adducts was less in human skin (Carmichael et al. 1991). The

complexity of DNA adduct formation was also shown to be less complex in human skin than in mouse skin following treatment *in vitro* with BaP (Watson et al. 1988). Thus, not only are the levels of DNA adducts less in human skin than in mouse skin, but the complexity of the DNA adduct profile is also less complex, indicating that fewer types of DNA adducts are formed in human skin than in mouse skin.

The results of an *in vitro* study conducted by Leadon et al. (1995) indicated that the total number of PAH-DNA adducts formed in isolated human mammary epithelial cells incubated with equal concentrations of nine different coal tar residues varied only by approximately 5-fold (total adducts ranged from 2 to 10 per 10^6 base pairs). Following incubation with BaP, only 20 total adducts per 10^6 base pairs were reported. However, the authors noted that the formation of adducts did not correlate with the PAH or the BaP content in the residues (total PAH contents in the residues ranged from 24,200 to 328,000 mg/kg, while BaP content ranged from 0.9% to 3.8%). Based on these results, the authors concluded that neither total PAH content nor BaP content was predictive of the level of DNA adducts formed following exposures to coal tar mixtures.

Godschalk et al. (1998) examined the applicability of DNA adducts in white blood cell (WBC) subpopulations as a measure of dermal exposure to PAHs in a group of eczema patients treated with coal tar ointment. Punch biopsies of the skin were performed to measure levels of DNA adducts in the skin following treatment. Urinary excretion of PAH metabolites, specifically 1-OH-P and 3-hydroxybenzo(a)pyrene (3-OH-B(a)P), was also monitored, and statistical analyses were conducted to determine if correlations between urinary metabolites and specific DNA adducts measured in WBC subpopulations or the skin existed. One week following treatment, increases in levels of aromatic DNA adducts were observed in the skin, and in monocytes and lymphocytes, with increases much greater in the skin. No significant correlation between urinary excretion of 1-OH-P or 3-OH-B(a)P and total DNA adducts in the skin was observed. However, a significant correlation ($p=0.025$) between urinary excretion of 3-OH-B(a)P and levels of benzo(a)pyrene-diol-epoxide-DNA in the skin was reported. The authors concluded that DNA adduct levels in mononuclear WBCs can possibly be used as a surrogate for skin DNA after dermal exposure to PAHs. However, the sensitivity of this measure of exposure

is limited. Furthermore, the authors indicated that measurements of urinary PAH metabolites seem to be more sensitive.

There is also evidence that neither the formation of DNA adducts nor the quantity of DNA damage in a tissue is predictive of carcinogenicity (Berton et al. 1997; Boerrigter et al. 1995; Goldstein et al. 1998; Harris 1987). In a study by Goldstein et al. (1998), tumor induction and the formation of DNA adducts following exposures to BaP and coal tars were compared. The authors reported that tumorigenesis did not correlate with either total adducts or adducts formed by BaP. Further, DNA adducts were identified in the lung, liver, and forestomach in mice that received BaP in the diet; however, tumors were only reported in the forestomach. The results of Boerrigter et al. (1995) indicated that following an intraperitoneal injection of 50 mg BaP/kg body weight in mice, DNA adducts were identified in the lung, liver, spleen, heart, brain and kidney, although BaP has not been shown to be carcinogenic in all of these tissues. Harris (1987) noted that DNA adducts were formed when aflatoxin B_1 was cultured with human bronchus, esophagus, colon and bladder tissues; however, aflatoxin has not been shown to be carcinogenic in these tissues. The authors concluded that carcinogenesis was a complex process and DNA damage was only a single aspect of that process. Hemminiki (1993) noted in a review paper on DNA adducts, mutations, and cancer that DNA adducts are formed in both target tissues and non-target tissues "indicating that adducts are not the only reason for tissue specificity," and that formation of DNA adducts from many compounds appears to be linear with a wide range of doses, regardless of the tumorigenic outcome. Meier and Washawsky (1994) also noted that DNA adducts resulting from topical application of BaP occurred in non-target tissues. Meier and Washawsky (1994) reported approximately 25 pmol/mg DNA in the liver 12 hours after topical administration of BaP to mice, but commented that BaP does not cause liver tumors. Yohn et al. (1988) noted that no tumors were observed in human xenografts following exposure to BaP diol-epoxide although DNA adducts were observed. The results of an experiment in two strains of mice, SENCAR and SKH-1, with different susceptibility to UV light induced skin tumors, indicated that there was an equal amount of DNA damage in the two strains following UV exposure, leading the authors to conclude that events other than DNA damage were responsible for UV-induced tumorigenesis in mouse skin (Berton et al. 1997). Collectively, these

studies provide evidence that the formation of adducts is insufficient for tumorigenesis and that other events must occur in addition to DNA damage for tumor formation to occur.

A second important consideration in the assessment of the formation of DNA adducts by PAHs is the potential species differences in the ability to repair the damaged DNA (Harris 1987). It has been suggested that the persistence of DNA adducts, due to slower repair rates, in mouse skin may account in part for the increased susceptibility of mice to the carcinogenic effects of BaP when compared to relatively resistant species such as the rat (Alexandrov et al. 1983). Alexandrov et al. (1983) reported that 3 weeks after dosing with BaP (250 nmol/mouse; 1000 nmol/rat), 7.3% (0.38 pmol/mg DNA) of the initial levels of BaP-DNA adducts (5.20 pmol/mg DNA) persisted in mouse skin, whereas, less than 0.5% (0.06 pmol/mg DNA)of the initial levels BaP-DNA adducts (12.36 pmol/mg DNA) was detectible in rats. Further, 6.5% of BaP diol epoxide adducts were present in mouse skin 3 weeks following dosing, while no BaP diol epoxide adducts were detected in rat skin 3 weeks after dosing, although the levels of these adducted were essentially the same (2.77 pmol/mg DNA in the mouse compared to 2.48 pmol/mg DNA in the rat). The findings in the mouse skin are supported by the results of Nakayama et al. (1984), where it was reported that after 14 days, 10% of the BaP-DNA adducts still remained in mouse skin after the application of 500 nmol BaP/mouse. Based on these results, Alexandrov et al. (1983) concluded that the longer retention time of DNA adducts due to the slower repair rates in the mouse compared to the rat could be the reason that the rat was more resistant to BaP carcinogenesis.

It is generally thought that DNA repair in human skin is more efficient than in mouse skin (Kuroki et al. 1989; Van Schooten and Godschalk 1996). In a study by Taichman and Setlow (1979), cultures of human keratinocytes and fibroblasts were exposed to UV-B light at a range of doses between 0-80 J/m^2, and the amount of UV-induced damage and repair were investigated. Both human keratinocytes and fibroblasts removed approximately 50% of the UV-induced pyrimidine dimers over a 16-hour period. Thus, the rates of DNA repair in HEKs and human dermal fibroblasts where equivalent following exposure to UV-B light and fibroblast repair rates could be used to estimate repair rates in epidermal cells. Consistent with this report, Gibson-D'Ambrosio et al. (1983) reported that fetal skin cell cultures removed 65% of pyrimidine dimers over a 24-hour period following UV irradiation (10 J/m^2).

In contrast, Bowden et al. (1975, 1977) reported that approximately 10% or 39% of the pyrimidine dimers were removed from mouse keratinocytes *in vitro* or mouse skin *in vivo*, respectively, over a 24-hour period following UV irradiation. In the study conducted by Bowden et al. (1975), the shaved backs of CD-1 mice were exposed to UV light for various times to up to 84,000 ergs/m^2; however, only the lowest exposure (4,200 ergs/m^2) had a sufficient number of observations for statistical comparisons to the unexposed controls. In the low-exposure group, excision of dimers was reported to be 39% at 24 hours post-exposure. In the Bowden et al. (1977) study, only 10% of the dimers induced following exposure to 10 J/m^2 UV light were repaired in mouse epidermal cells *in vitro*. Comparing the percent values of DNA repair reported for mouse skin with the values reported for human skin, human skin repaired up to 5-fold more UV-DNA damage than mouse skin in a 24-hour period.

Reports in the literature have shown that human skin cells are capable of repairing between 60% and 100% of the BaP adducts formed within the first 24-48 hours following exposure to *anti*-BPDE (BaP diol epoxide). In a study conducted by Day et al. (1978), human fibroblasts were incubated in the presence of *anti*-BPDE (3.3 or 16.5 Fg/ml) for 30 minutes, after which the carcinogen containing media was removed and replaced with fresh media. Normal human fibroblasts treated with *anti*-BPDE (3.3 Fg/ml = 1 µM) repaired 85% of the diol-epoxide DNA adducts within 4 hours following exposure. Further, almost 100% of the diol-epoxide DNA-adducts had been removed within 24 hours following exposure. Approximately 40% of the adducts were removed within 2 hours in cells incubated with 16.5 Fg/ml, with approximately 55% remaining after 24 hours. Van Houten et al. (1986) also reported on DNA repair in human fibroblasts treated with 4 µM BPDE for 1 hour. Approximately 60% of the BaP-DNA adducts were removed in the first 24 hours following exposure to BPDE; and 80% of the BaP-DNA adducts had been repaired by 48 hours following the treatment (Van Houten et al. 1986).

Mouse skin has also been reported to remove BaP-DNA adducts; however, the rate of removal appears to be considerably slower than the rate observed in human cells. In a study conducted by Nakayama et al. (1984), primary BALB/c epidermal cells prepared from newborn mice were cultured in 1 µM *anti*-BPDE for one hour. Following the incubation, the media was replaced with fresh media, and the cells were harvested and examined for BaP-DNA adduct formation at various time points from 0-48 hours. Mouse cells removed 55% of the BaP-DNA

adducts in the first 24 hours; however, no noticeable removal was evident between 24 and 48 hours. Thus, the total removal of BaP-DNA adducts over a 48-hour period (55-60%) in mice was considerably less than that observed for human cells (80-100%).

In summary, the results of the studies where DNA adduct formation has been studied in mouse and human skin indicated that at presumably comparable doses of coal tar ointment, the levels of DNA adducts formed in human skin were approximately 2-fold less than levels formed in mouse skin. In mouse skin, the adducts formed tended to be more complex than in human skin. Further, the total PAH content or the amount of BaP in a mixture did not correlate with the amount of DNA adduct formation. Moreover, following exposure to BaP, DNA adducts have been shown to form in tissues other than the target tissue(s), indicating that adduct formation alone was insufficient and that some other event or events were required for tumor formation. Also, the repair of DNA adducts was faster in human cells, when compared to mouse cells. Collectively, these data indicate that DNA adducts represent a biomarker of exposure and that the formation of adducts alone is not enough to result in tumor formation, i.e., some other events must occur in addition to adduct formation. Repair of DNA adducts appears to be faster in human skin cells than in mouse skin cells, with DNA adducts persisting for a longer period of time in mouse skin cells suggesting that mouse skin may be more sensitive to the effects of PAHs than human skin.

2.4.2.2 Comparison of Tumor Promotion

Coal tar is a complex mixture containing more than 10,000 constituents, and it is known that some of the constituents in coal tar, such as BaP and DMBA, possess both initiation (form DNA adducts) and promotion abilities (Boutwell 1978; O'Brien 1976), while other constituents may only promote initiated cells (Mahlum 1983). It has been demonstrated that either a single large dose or smaller repetitive doses of a carcinogenic PAH, such as DMBA, can produce skin tumors without subsequent promotion (Boutwell 1976). However, the ability to be a "complete" carcinogen is entirely dose-dependent as demonstrated by O'Brien (1976). At the doses that result in skin tumors without subsequent promotion, several PAHs were found to have both initiating and promoting capabilities, while at lower doses, the same PAHs were found to have

only initiating capabilities, i.e., the dose was sufficient to result in DNA adduct formation with no subsequent tumor development.

A two-stage initiation/promotion model for the formation of skin tumors in mouse skin has been developed. In this model system, the first step, initiation of the epidermal cells, is achieved by the application of an initiating dose of a chemical, such as DMBA, that would result in DNA damage but would not result in tumor formation (Verma et al. 1980a). The second step consists of repeated applications of a tumor promoter, such as TPA, to the initiated skin (Verma et al. 1980a). The application of tumor promoters to initiated skin results in the clonal expansion of the initiated cells and subsequent tumor development in mouse skin (Marks et al. 1999; Yuspa 1994). This two-stage model has been used extensively to study tumorigenesis in mouse skin.

Promotion involves a complex cascade of events and a consensus on the precise sequence of events that occur in mouse skin has not been reached in the scientific literature. However, there is a considerable amount of data that has been collected from which some conclusions can be drawn regarding the sequence of events that lead to promotion in mouse skin. Specifically, the evidence suggests that promotion in mouse skin is a prostaglandin-dependent process that requires the induction of ODC and the activation of AP-1 (Ashendel and Boutwell 1979; Cameron et al. 1992; Fischer et al. 1993; Furstenberger and Marks 1978; Jansen et al. 1999; Kennard et al. 1995; Suda et al. 2000; Verma et al. 1977, 1988; Young et al. 1999). In contrast, as discussed below, the evidence suggests that the promotional pathways in human skin are prostaglandin independent (Arnold et al. 1990, 1992; Cameron et al. 1992).

Tumor Promotion in Mouse Skin

In mouse skin, the proposed mode of TPA tumor promotion is shown in Figure 2-2 and can be summarized as follows. Tumor promotion is thought to involve a cascade of events that is initiated by the interaction of TPA with PKC as shown in Figure 2-2 (Corsini et al. 1990; Takigawa et al. 1983; Verma et al. 1986, 1988). This activates cyclooxygenase 2 (COX2), which increases the formation of prostaglandin E2 (PGE2) from arachidonic acid (Cameron et al. 1992; Verma et al. 1988). The formation of PGE2 initiates two pathways: 1) induction of ODC activity (Ashendel and Boutwell 1979; Furstenberger and Marks 1978; Verma et al. 1977) and 2) activation of c-*jun*, c-*fos* and AP-1 (Fischer et al. 1993; Kennard et al. 1995; Suda et al. 2000;

Young et al. 1999). The activation of both the ODC and AP-1 pathways is necessary for tumor promotion by TPA in mouse skin (Jansen et al. 1999). These steps are discussed further in the following paragraphs.

TPA is thought to exert its effects through PKC in that it interacts with a membrane receptor that promotes the translocation of PKC from the cytosol to a membrane-bound state (Corsini et al. 1990; Verma et al. 1986, 1988). The binding of PKC to TPA was dependent on the presence of calcium and phospholipids and resulted in activation of the enzyme, while simultaneously down-regulating the amount of free PKC in the cytosol. Once activated, PKC mediates the phosphorylation of cytosolic proteins, leading to an increase in the synthesis and release of prostaglandins, such as PGE2 via the action of COX2 (Cameron et al. 1992; Verma et al. 1988) and products of the lipoxygenase pathway (Aizu et al. 1986). The importance of the activation of PKC in the two-stage model of skin carcinogenesis in mice was demonstrated by Takigawa et al. (1983) and Yamada et al. (1988). In these studies, the inhibition of PKC by %-difluoromethylornithine (DFNO) or staurosporine in CD-1 mice resulted in decreases in TPA-induced ODC activity and decreases in tumor formation.

It has been demonstrated that TPA increases the synthesis of PGE2 in mouse skin (Ashendel and Boutwell 1979; Muller-Decker et al. 1998; Ordman et al. 1985; Verma et al. 1977; 1980b). A single application of TPA (17 nmoles) to CD-1 mice resulted in about an 8-10 fold increase in PGE2 synthesis (Ashendel and Boutwell 1979). The synthesis of PGE2 is essential for the induction of ODC (Ashendel and Boutwell 1979; Furstenberger and Marks 1978; Verma et al. 1977), which has long been recognized to play a role in TPA tumor promotion and is considered an important biomarker associated with TPA-mediated tumor promotion (Boutwell 1978; O'Brien 1976). In addition to ODC induction, the evidence suggests that the expression of c-*jun* and c-*fos* and subsequent activation of AP-1, which also are likely necessary in tumor promotion in mouse skin, are activated in response to PGE2 (Suda et al. 2000; Young et al. 1999).

The application of COX2 inhibitors has been shown to decrease ODC induction in mice (Loprinzi and Verma 1985; Nakadate et al. 1982; Ordman et al. 1985; Verma et al. 1977, 1980b). When treated with the COX2 inhibitors indomethacin, flufenamic acid, or acetylsalicylic acid

prior to TPA application, ODC activity was decreased by approximately 3- to 4-fold, when compared to the vehicle control mice (Verma et al. 1977, 1980b). However, when PGE1 or PGE2 were administered concurrently with TPA to mice pretreated with indomethacin, the effects of indomethacin were reversed and ODC activity was comparable to the vehicle control. PGF1% or PGF2% did not reverse indomethacin inhibition of ODC induction. These findings are supported by the results of Ordman et al. (1985) where the application of indomethacin decreased TPA-induced ODC activity by 15-fold in CD-1 mice. This effect was reversed by the application of PGE. Further, pretreatment with indomethacin at doses that resulted in a 4-fold decrease in ODC activity, decreased the total number of tumors/mouse by approximately 50% in a DMBA/TPA initiation/promotion assay, and the percentage of mice with tumors was further decreased by approximately 6% after treatment with indomethacin (Verma et al. 1980b). Based on these results, Verma et al. (1980b) concluded that prostaglandins play a role in ODC induction and subsequent tumor formation in mouse skin. Muller-Decker et al. (1995) reported that the concurrent application of TPA (5 nmol) and indomethacin (0.56 Fmol) to the DMBA-initiated skin of NMRI mice decreased tumor incidence by 66%, when compared to the tumor incidence in mice that received TPA alone. This effect was reversed by the application of 30 nmol of PGE2 or PGF2%, which resulted in an increase in tumor incidence. Similar results were reported by Furstenberger et al. (1989).

In addition to activation of the COX2 pathway, there is evidence that TPA can activate the lipoxygenase prostaglandin synthesis pathway and that the activation of this pathway was involved in the induction of ODC activity (Aizu et al. 1986). In a study in female CD-1 mice, the application of the lipoxygenase inhibitors 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone or 3,4,2',4'-tetrahydroxychalcone concurrently with TPA to DMBA-initiated skin decreased peak ODC induction by approximately 3-fold (Aizu et al. 1986). Further, the incidence of skin tumors was decreased by approximately 20% and 50% in mice that were treated with 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone or 3,4,2',4'-tetrahydroxychalcone, respectively.

There is evidence that PAHs, components of coal tar, can induce ODC in mouse skin, and that the induction of ODC by PAHs is prostaglandin-dependent (Andrews et al. 1991a; Nakadate

et al. 1989; Yamamoto et al. 1992). A single application of 200 nmoles of the PAH, 7-bromethylbenz[a]anthracene (BrMBA), a structural analog of DMBA, to skin of CD-1 mice resulted in an approximate 25- to 27-fold increase in ODC activity (Nakadate et al. 1989; Yamamoto et al. 1992). However, the application of indomethacin + BrMBA resulted in ODC induction only approximately 7-fold greater than the vehicle controls (Yamamoto et al. 1992). The application of the lipoxygenase inhibitors nordihydroguaiaretic acid (10 Fmol), 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (10 Fmol), or quercetin (3 Fmol) inhibited BrMBA-induced ODC activity by 15-fold, 0.5-fold and 9-fold, respectively (Nakadate et al. 1989). Further, the application of quercetin (10 Fmol) resulted in a significant decrease in the incidence of papillomas in CD-1 mice initiated with DMBA and promoted with BrMBA (Nakadate et al. 1989). Other studies have shown that chemicals, such as ginger extract and isoliquiritigenin, that inhibit prostaglandin synthesis also inhibited TPA-induced ODC activity and tumor formation (Katiyar et al. 1996; Yamamoto et al. 1991).

The application of 0.1 Fg (twice weekly for 3 weeks) BaP to the skin of BALB/c mice resulted in a 7-fold (statistically significant) increase in PGE2 levels (Andrews et al. 1991a). When BaP was applied to mouse skin in areas where subcutaneous implants that delivered 2.4 Fg indomethacin/day had been placed, the PGE2 levels observed following the application of BaP were statistically significantly decreased, when compared to PGE2 levels observed in skin that had not received indomethacin implants. Further, the mean week of tumor onset was statistically significantly decreased in mice that were treated with BaP + indomethacin (twice weekly for 6 months), when compared to mice the received BaP alone (Andrews et al. 1991b).

The application of DMBA (1% in cream twice weekly for 3 weeks to 6 months) did not significantly increase PGE2 levels in mouse skin. No appreciable alterations in PGE2 levels or in the mean week of tumor onset was noted with indomethacin implants delivering 2.4 Fg indomethacin/day (Andrews et al. 1991a,b).

Ordman et al. (1985) reported that the application of DMBA to CD-1 mouse skin 42 hours prior to sacrifice [peak ODC activity following DMBA application has been reported at approximately 36 hours (Verma et al. (1980a)] resulted in an approximate 3-fold increase in ODC levels over the vehicle controls. When indomethacin (100 Fg) was applied 2 hours prior to

DMBA (44 hours prior to sacrifice), ODC activity was decreased by 20%. In contrast, application of the lipooxygenase inhibitor, quercetin, significantly reduced the incidence of papillomas in CD-1 mice treated with DMBA (Nakadate et al. 1989). Consequently, DMBA may be a strong initiator but a weak tumor promoter and that promotion may be lypooxygenase-mediated rather than COX-mediated.

Collectively, these data demonstrated that increased levels of ODC activity in mouse skin were induced by prostaglandins and that the application of prostaglandin inhibitors decreased ODC induction. Further, inhibition of prostaglandin synthesis also resulted in decreased ODC activity with TPA and with the PAHs, BrMBA, BaP, DMBA. Thus, in mouse skin, ODC induction is a prostaglandin-dependent process that is responsive to both TPA and PAH induction. Further, a single application of DMBA, 3-MC, BaP, or 1,2,5,6-dibenzanthracene applied at a dose (3.6 Fmoles/mouse) high enough to result in initiation and promotion of cells, caused induction of ODC, a biomarker of promotion, similar to that achieved with the mouse tumor promoter TPA (O'Brien 1976). When low doses capable only of initiation were applied (0.1 µmoles/mouse), none of the compounds tested caused an increase in ODC any time after treatment. Therefore, the development of mouse skin tumors following exposure to constituents in coal tar such as DMBA and BaP appears to be a two-stage process with both initiation and promotion necessary.

There are many other studies that provide evidence that TPA induces ODC activity in mice epidermal cells *in vivo* and *in vitro* (Chida and Kuroki 1984; Cope et al. 1988; Corsini et al. 1990; Fischer et al. 1989; Halmekyto et al. 1992; Jansen et al. 1999; O'Brien 1976; Yamamoto et al. 1988) and increases in ODC mRNA (Kennard et al. 1995). Dose-related increases in ODC activity were observed following a single application of TPA (100 nmoles) to C57BL/6, CD-1 or SENCAR mice, with the maximum increases about 5- to 15-fold greater than baseline levels (Yamamoto et al. 1988). Cope et al. (1988) reported dose-related increases in ODC activity in CD-1 mice following TPA application with the maximum increase approximately 130 times higher than the baseline level (TPA applied at a dose of 11 µg/mouse). Fischer et al. (1993) reported that in cultured mouse keratinocytes, TPA (0.1 µg/ml) induced ODC activity by at least 10-fold, while Chida and Kuroki (1984) reported that TPA (1 µg/ml) induced ODC activity

about 8.5-fold over basal levels also in cultured mouse epidermal cells. In a study by Kennard et al. (1995), ODC mRNA was induced by about a factor of about 9- to 13-fold in SSIN mice (2 µg TPA/mouse) and about 10- to 16-fold in C57BL/6 mice (4 µg/mouse) over basal levels after the application of TPA. Thus, the evidence indicates that the mouse is very sensitive to ODC induction by TPA and depending on the experimental conditions, the application of TPA can increase ODC activity in mouse skin by up to 130-fold.

In mouse skin, the induction of ODC initiates a series of events that result in increased DNA and polyamine synthesis (Chida and Kuroki 1984; O'Brien et al. 1989). Polyamines have been shown to play a role in DNA synthesis and cell proliferation (Niggli and Rothlisberger 1988; Paulsen and Astrup 1983). Following TPA (17 nmoles) application to CD-1 mice, the polyamines spermine, spermidine and putrescine increased by approximately 2-fold, 4-fold and 10-fold, respectively (O'Brien 1976). The peak in ODC activity was 4.5 hours after TPA application, prior to the peak increases in polyamine synthesis, which were at 24, 72 and 7 hours after TPA application for spermine, spermidine and putrescine, respectively. Concentration-related increases in DNA synthesis have also been shown to increase following TPA application in isolated mouse epidermal cells cultured with TPA (Chida and Kuroki 1984). Increases in DNA synthesis were observed with TPA concentrations ranging from 0.1 to 10 ng/ml. Concentration-related increases in ODC activity were also induced at these concentrations of TPA. The maximum increase in DNA synthesis, about 1.5-fold greater than baseline, was observed with 10 ng TPA/ml. At higher concentrations, DNA synthesis was inhibited.

Based on the data available at the time, O'Brien (1976) and Boutwell (1978) proposed that in mouse skin promotion induces epidermal cells to the tumor phenotype. In initiated cells, an initiation-induced error prevents the cell from repairing and reverting to the normal phenotype and the initiation-induced error alters the system that controls ODC. Consequently, elevated ODC levels are sustained leading to increased synthesis of polyamines and a greater potential for growth. Normal, uninitiated cells are capable of repairing the error and the ODC levels after an initial spike return to basal levels. Thus, based on the model of tumor promotion proposed by O'Brien (1976) and Boutwell (1978), ODC induction is an obligatory precursor step to tumor promotion. Further, O'Brien (1976) and Boutwell (1978) noted the distinction between

promotion and hyperplasia in that promotion induces proliferation or clonal expansion of initiated cells, while hyperplasia was defined as an increase in the number of normal or noninitiated cells. O'Brien (1976) and Boutwell (1978) indicated that although TPA elevates the levels of ODC and S-andenosylmethionine decarboxylase (SAM) promotion was mediated by ODC, while hyperplasia was mediated by SAM. Boutwell (1978) noted that acetic acid increased protein and DNA synthesis and hyperplasia, but did not induce promotion. SAM levels were increased following the application of acetic acid, which is an irritant to human skin; however, ODC activity was not induced in mouse skin *in vivo*. Thus, Boutwell (1978) concluded that although both ODC and SAM may be elevated in hyperplastic skin, the induction of SAM was related to hyperplasia, while ODC induction was necessary for tumor promotion.

Recent evidence suggests that in addition to the elements described by O'Brien (1976) and Boutwell (1978) as essential for tumor promotion in the mouse skin, the expression of c-*jun* and c-*fos* and the subsequent activation of AP-1 also play an important role in the promotion process (Jansen et al. 1999; Young et al. 1999). As illustrated in Figure 2-2, increased levels of PGE2 also increase c-*jun* and c-*fos* expression, the primary mediators of TPA transcription products and which also activate AP-1 (Fischer et al. 1993; Kennard et al. 1995; Suda et al. 2000; Young et al. 1999). Kennard et al. (1995) demonstrated that a single application of TPA to SSIN (2 µg/mouse) or C57BL/6 (4 µg/mouse) mice induced c-*fos* mRNA more than 50-fold in each strain. c-*jun* mRNA was also induced, although the response was somewhat weaker with only a 2- to 4-fold increased observed. The application of 8 nmol of TPA to the skin of CD-1 mice resulted in a 6-fold increase in c-*fos* mRNA (Oguro et al. 1998). TPA (dose not reported) also induced an approximate 30-fold increase in c-*fos* RNA in cultured mouse primary keratinocytes *in vitro* (Dotto et al. 1986). Although data were unavailable for mouse epidermal cells, Suda et al. (2000) demonstrated that PGE2 induced c-*jun* and c-*fos* in a dose-dependent manner in osteoblasts. Maximal induction was approximately 3-fold and 10-fold for c-*jun* and c-*fos*, respectively. Thus, the experimental data indicate that TPA can induce c-*jun* and c-*fos* expression and that the trigger for these events may be via a PGE2 mediated pathway.

The results of Young et al. (1999) indicated that transactivation of transcription factor activator protein (AP-1), which is activated by c-*jun* and c-*fos*, was required for tumor

promotion. In this study, transgenic mice with the TAM67 transgene, which inhibits AP-1, were used. When tested in a DMBA/TPA initiation/promotion protocol (400 nmole DMBA followed by 10 nmole TPA twice weekly for 20 weeks), expression of TAM67 blocked AP-1 activation. Further, this was associated with an approximately 80% decrease in TPA-promoted tumor formation, when compared to nonstrangenic mice. However, proliferation and ODC induction were observed following TPA application in the transgenic line. The authors concluded that AP-1 transactivation was necessary for tumor promotion and that proliferation and the induction of ODC alone were insufficient for tumor promotion.

The results of Young et al. (1999) are supported by the results of Jansen et al. (1999), where it was reported that in the mouse JB6 epidermal cell line, TPA induced ODC activity and activated AP-1; however, the activation of AP-1 was not dependent of induction of ODC. When incubated with difluoromethylornithine (DFNO), an irreversible inhibitor of ODC, cell transformation, determined by the number of colonies capable of anchorage independent growth, did not occur. However, DFNO did not alter AP-1 activity. In cells that expressed dominant c-*jun*, which inhibits AP-1 activation, the induction of ODC by TPA was unchanged but cell transformation did not occur. Based on these results, the authors concluded that the induction of ODC and the activation of AP-1 by TPA were independent events and that both were necessary for cell transformation.

Comparison of Mouse and Human Response to Complex PAH Mixtures

The potential for complex PAH-containing mixtures to induce ODC activity was evaluated by Swanger et al. (1983) and Gupta and Mehrotra (1990). In the study by Swanger et al. (1983), the induction of ODC activity in mouse skin by subfractions of a coal tar creosote, identified as neutral tar, basic tar and the PAH fraction, obtained from solvent-refined coal processes, was investigated. ODC activity following the application of each of these subfractions to the skin of CD-1 mice was 3.5-, 2.8-, and 5-fold higher for the neutral tar, basic tar and PAH subfractions, respectively, than ODC activity in the vehicle (acetone) controls. The application of 50 Fl of either fresh or used cutting oil, another complex mixture that contains PAHs, to the skin of Swiss albino mice resulted in a 5- to 6-fold increase in ODC activity (Gupta and Mehrotra

1990). These data indicate that in mouse skin, the application of complex PAH-containing mixtures induce ODC activity.

In contrast, application of pharmaceutical grade coal tar did not induce ODC activity in human skin. A study conducted by Arnold et al. (1993) investigated the effects of pharmaceutical grade coal tar on ODC activity in human skin. In this study, ODC activity was measured in skin biopsies collected from human volunteers with normal skin and from psoriasis patients. Pharmaceutical grade coal tar was applied to psoriatic or normal skin and covered with gauze. After 16 hours, the areas were tape-stripped and a second application of coal tar was applied and 8 hours later, biopsies were taken. In addition, two volunteers received coal tar applications twice daily for one week, similar to the protocol used to treat psoriasis, followed by tape-stripping and biopsy. Additional sites from each group received placebo treatments. ODC activity in the volunteers who received coal tar or in the psoriasis patients was not statistically significantly different from the placebo treated areas. In fact, ODC activity was slightly decreased (21%) in the coal tar-treated areas, when compared with ODC activity in the placebo-treated areas. Further, in the two individuals who received twice daily applications of coal tar, there were no changes in ODC activity. These data provide evidence that the application of pharmaceutical grade coal tar does not increase ODC activity in human skin.

Comparison of Mouse and Human Response to TPA and UV Light

Human skin keratinocytes may also be promoted, although the experimental evidence suggests that the pathway through which promoters act in human skin differs from mouse skin and the magnitude of that promotion differs (Chida and Kuroki 1984; Corsinsi et al. 1990; Fischer et al. 1993). In mouse, application of 0.95, 1.9 or 11.0 µg TPA to shaved mouse skin for 4.5 hours resulted in a dose-dependent increased ODC activity in the harvested skin (Cope et al. 1988). In this study, the activity increased more than 100-fold at the highest TPA dose (from 0.002 to 0.22 nmole CO_2 /min/mg protein (Cope et al. 1988). In contrast, when human skin punch biopsy specimens from amputated human skin or human volunteers were incubated with 1 µM TPA in ethanol for various time periods, peak ODC induction occurred at 6 hours and resulted in a modest 2- to 5-fold increase over the vehicle control (Loprinzi and Verma 1984;

Loprinzi et al. 1985; Verma et al. 1985), when measured as pmoles CO_2 /hr/biopsy sample. However, this increase was slightly less than 2-fold when expressed as nmoles CO_2 /min/mg of protein with values of 0.038 nmoles CO_2 /min/mg of protein reported in ethanol vehicle control and 0.068 nmoles CO_2 /min/mg of protein reported in the TPA in ethanol-treated biopsy samples (Verma et al. 1985). In contrast, following tape-stripping of the skin of healthy human volunteers, a greater than 100-fold increase in ODC activity over baseline was reported (Arnold et al. 1992). Thus, the ODC response of human skin to TPA, if any, was marginal.

Verma et al. (1985) noted that based on the method of isolation of the epidermal layer, it was not possible to conclude from the results which cell type in the human epidermis was induced. The punch biopsy technique used by this group is intended to remove small 3 mm diameter samples from the epidermis. However, some of the dermis is removed as well in these biopsies. The dermis contains cells such as fibroblasts that have been shown to be a source of ODC (Niggli and Rothlisberger 1988) and it has been reported that TPA up regulated gene expression in human fibroblasts *in vitro* (Soriani et al. 1999). It is possible that a small fraction of fibroblasts may have been present in the samples tested by Verma and colleagues and that these fibroblasts may have been the source of the observed changes in ODC.

Studies in isolated human keratinocytes indicate that TPA did not induce ODC in these cells. Fischer et al. (1993) suggested that the downstream consequences of PKC activation was opposite in mouse skin and human skin. As discussed above, in isolated mouse keratinocytes, activation of PKC by TPA (1 µg TPA/ml) resulted in the induction of ODC (approximately 10-fold) and expression of c-*jun* and c-*fos*, steps that were necessary for promotion in mouse skin. However, in isolated human keratinocytes, activation of PKC by TPA decreased ODC mRNA and did not alter c-*jun* and c-*fos* mRNA (Fischer et al. 1993). Thus, events that appear to be necessary for tumor promotion in the mouse did not occur in human keratinocytes in response to TPA.

Similar observations have been reported by other groups investigating the differential effects of tumor promoters in mouse and human keratinocytes (Corsini et al. 1990; Fischer et al. 1984). Corsini et al. (1990) reported that TPA (1 µmole) treatment inhibited basal ODC activity, significantly decreased thymidine incorporation and cell proliferation in the human epidermal

NCTC cell line. The opposite was observed in the murine HEL-30 epidermal cell line, where TPA (1 µmole) induced increases in ODC activity and cell proliferation (Corsini et al. 1990). The addition of the specific PKC inhibitor, H-7, blocked these effects in murine epithelial cells. Based on these results, Corsini et al. (1990) concluded that activation of PKC induced cell proliferation in mouse epidermal cells, but not in human cells.

TPA treatment decreased ODC activity by 50-90% in cultured human keratinocytes; however, there were no effects on ODC mRNA levels (Ruhl et al. 1994). The authors suggested that the suppression of ODC activity by TPA (100 ng/ml) in human keratinocytes was due to a TPA-mediated decrease in ODC mRNA translatability.

Chida and Kuroki (1984) reported a dose-related decrease in DNA synthesis and D-glucose uptake, and no increase in ODC activity in HEKs incubated with 0.1 to 1000 ng TPA/ml, whereas, a dose-related increase in all these activities was observed in murine epidermal keratinocytes. Thus, the results of these studies indicate that TPA does not activate the series of events in isolated human keratinocytes that result in increased ODC activity and polyamine synthesis and activation of AP-1 in mouse skin, events that are likely necessary for promotion in mouse skin.

UV-B light has been shown to function as a complete carcinogen in mice, possessing both initiation and promotion abilities (Berton et al. 1997; Lowe 1981). Berton et al. (1997) reported that the carcinogenic effect of UV-B light was correlated with epidermal proliferation, but not DNA photodamage, and thus, highlighted the importance of tumor promotion in the development of tumors in mice exposed chronically to UV-B light. As seen with TPA, UV-B light stimulated ODC activity, and DNA and polyamine synthesis in mouse skin (Hillebrand et al. 1990; Lowe 1981; Lowe et al. 1982; Nemoto et al. 1986). Nemoto et al. (1986) reported an approximate 2-fold increase in ODC activity in mice following a single exposure to UV-B. In mice exposed to UV-B three times a week for 27 weeks, a 350-fold increase in ODC activity was reported (Hillebrand et al. 1990). Further, as with TPA, in mouse skin, UV-B mediated induction of ODC was regulated by prostaglandins (Lowe 1981; Lowe et al. 1982). Pretreament with indomethacin resulted in about a 2- to 2.5-fold decrease in UV-B-induced ODC activity, while triamcinolone decreased ODC activity 4- to 5-fold (Lowe 1981; Lowe et al. 1982).

Polyamine synthesis was also increased following UV-B exposure in hairless mice (Lowe 1981). Following single exposures to UV-B, putrescine levels were reported to parallel ODC activity, with a maximum peak of approximately 13-fold over baseline, with decreases of 10% and 70% in spermidine and spermine levels reported, respectively. Following multiple exposures to UV-B, putrescine, spermine and spermidine were all significantly increased. The application of indomethacin resulted in slight a decrease (17%) in DNA synthesis following UV-B exposure, while triamcinolone decreased DNA synthesis by 75% (Lowe 1981). Thus, as with TPA, the experimental evidence suggests that in mouse skin, following UV-B exposures, polyamine synthesis and DNA synthesis were likely induced by a prostaglandin-mediated pathway. From these data it can be concluded that in the mouse, chemical promotion with TPA or promotion with UV-B is via a PGE2-mediated pathway.

UV-B light has also been shown to induce ODC activity in human skin (Arnold et al. 1991, 1992; Niggli and Rothlisberger 1988); however, in contrast to the mouse, the induction of ODC by UV light appears to be prostaglandin independent. Arnold et al. (1991, 1992) reported no significant differences in the induction of ODC in the skin of human volunteers that were pretreated with topical indomethacin, when compared with the vehicle controls. The authors concluded that the induction of ODC by UV-B in human skin was not dependent on prostaglandins.

ODC induction in response to tape stripping provides additional evidence that the induction of ODC in human skin was not prostaglandin-dependent (Arnold et al. 1990, 1991, 1992). Arnold et al. (1990) measured ODC activity in the skin of normal healthy volunteers and from the uninvolved skin of psoriasis patients at various time points after tape stripping (tape stripping simulates skin “wounding”). ODC activity increased in the skin from each group, with the maximum increase approximately 80 times higher than activity immediately after tape stripping. There were no differences in ODC response between the nonpsoriasis group and the psoriasis patients. Oral indomethacin taken prior to tape stripping had no effect on the induction of ODC. However, topical betamethasone decreased ODC activity by about 50%. In a second study Arnold et al. (1991, 1992) noted that topical indomethacin had no effect on the induction of ODC activity in human skin by tape stripping. Based on the results of these studies Arnold et

al. concluded that the signal transduction pathways that result in ODC induction in human skin were not prostaglandin dependent, i.e., increased levels of prostaglandin were not necessary for the activation of this pathway. Thus, induction of ODC with UV-B, as with tape stripping, is likely through a prostaglandin-independent pathway in human skin, which is in contrast to promotion in mouse skin which is through a prostaglandin-dependent pathway.

The conclusions reached by Arnold et al. (1990, 1991, 1992) are supported by the results of Cameron et al. (1992) and DeLeo et al. (1986) where it was demonstrated that TPA (#1 Fg/ml) did not increase PGE2 or PGF2% *in vitro* when incubated with HEKs isolated from skin obtained from adult surgical specimens. In contrast, TPA (1 Fg/ml) increased PGE2 by approximately 3.5-fold in cultured mouse epidermal keratinocytes (MEKs) under the same assay conditions (Cameron et al. 1992).

2.4.2.3 Summary of Tumor Promotion

In summary, tumor promotion involves a complex series of biochemical events. In mouse skin, TPA tumor promotion was initiated in response to the interaction of TPA with PKC. Activation of PKC results in an increase in arachidonic acid metabolism and increased PGE2 synthesis, which induces ODC activity. The induction of ODC activity is an important step, and thought to be an obligatory precursor event, in the tumor promotion process in mice. Moreover, the induction of ODC has been observed following exposure to UV-B light and the application of PAHs to mouse skin, which suggests that in the mouse skin these agents induce ODC via the same prostaglandin-dependent biochemical pathway as TPA. Induction of ODC is associated with increased synthesis of polyamines, which are essential for DNA synthesis and cell proliferation. Further, TPA activated AP-1, which was necessary for promotion in transgenic mice, and the activation of AP-1 was via a prostaglandin-mediated pathway. Therefore, in mouse skin the initiation of the two critical events for tumor promotion, induction of ODC and activation of AP-1, was mediated through a prostaglandin-dependent pathway.

In contrast, TPA had no effect on ODC activity, DNA synthesis was decreased and the expression of c-*jun* and c-*fos*, which activate AP-1 in mouse skin, was not altered in human keratinocytes incubated with TPA *in vitro*. Moreover, both UV-B light and tape stripping

induced ODC activity in human skin; however, indomethacin had no effect on ODC activity induced by UV-B or tape stripping, indicating that the induction of ODC by these pathways in human skin was likely by a prostaglandin-independent pathway. Further, the application of pharmaceutical-grade coal tar to the skin of human volunteers did not increase ODC.

The difference in response observed between mouse and human skin can be attributed to the mechanism by which ODC activity is induced in the mouse and human skin. Specifically, the cascade that leads to induction of ODC activity in mouse skin following TPA promotion or following exposures to a PAH or UV-B, is a prostaglandin-dependent process, based on the results of experiments that indicated that indomethacin, which inhibits prostaglandin synthesis, also inhibited ODC synthesis. In contrast, induction of ODC activity in human skin by TPA, UV light or tape stripping was not a prostaglandin-dependent process and was not influenced by inhibitors of prostaglandin synthesis such as indomethacin. Therefore, the mechanism through which TPA promoted tumors in mice, the prostaglandin-mediated induction of ODC activity and activation of AP-1, was not operative in humans. Rather, ODC induction was by a prostaglandin-independent mechanism, as with the guinea pig, a species in which the mechanism for ODC induction was also a prostaglandin-independent process and a species that is resistant to TPA tumor promotion (Bourin et al. 1982; Prunieras et al. 1982).

The differences in response to TPA promotion between mouse skin and human skin may explain why tumors were produced in mouse skin following initiation with DMBA and promotion with TPA or repeated applications of BaP, but tumors were not observed in human skin xenografts following either of these treatments. Tumors in human xenografts were induced with either UV-B light alone or to a slightly increased rate when DMBA was followed by UV-B light. In the context of a multistage carcinogenic process, initiation, as might be expected with DMBA, did not progress to tumors in human skin without the additional promotional signal provided by UV-B light. In conclusion, the evidence suggests that the mode of tumor promotion in mouse skin is different than in human skin, and thus, human skin would be expected to be much less sensitive, if at all, to tumor promotion by TPA or PAHs, such as those found in coal tar. Due to these and other differences discussed in this report, the mouse skin carcinogenesis

model is not predictive of health outcomes in humans exposed to coal tar and is inappropriate for use in human health risk assessment.

2.5 Relevance to Human Health

In summary, animal models of skin carcinogenesis have made important contributions to our understanding of the biological principals of human skin cancer development (Yuspa 1994). These models, typically a multistage model in which a DNA-damaging dose or initiating dose of one of the PAHs, most typically DMBA, are followed by a mitogenic, tumor promoter, such as TPA, have been used extensively to study skin carcinogenesis for chemical agents. Mouse skin tumor models have defined molecular targets of tumor initiation, biochemical pathways in promotions of premalignant lesions, and intracellular mechanisms of tumor promotion in murine squamous carcinomas (Yuspa 1994). However, despite the widespread application and common acceptance of the assumptions made to allow extrapolations to humans, the relevance of murine skin cancer models to be quantitatively predictive of human skin cancer risk has not actually been demonstrated (Soballe et al. 1996). Application of these findings to humans must consider the important differences between the mouse and human skin (Boutwell 1978; Chida and Kuroki 1984; Soballe et al. 1996; Yuspa 1994). These differences, as discussed in detail in this report, have a profound influence on tumor development and render human skin much more resistant to skin tumor development when exposed to constituents in coal tar than when such chemicals come in contact with mouse skin.

Based on a weight-of-evidence evaluation of relevant primary and secondary data in mice and humans, the data clearly indicate that data from mouse skin painting studies are not appropriate for estimating potential risks of skin cancer in humans following dermal exposure to coal tars for the following reasons.

- No mouse skin painting study that assessed pharmaceutical grade coal tar was suitable for dose-response modeling. All but one of the mouse skin painting studies that evaluated other coal tar-derived mixtures suffered from one or more of the following limitations: tested at only one application level, applied only one application followed by chronic

application of a tumor promoter, had no control group, did not identify the dose level, or did not identify the coal tar mixture tested. Further, the only study with dose-response data tested creosote and the results were confounded by the severe ulceration produced by components in the mixture.

- Pharmacokinetic (AHH metabolism) and pharmacodynamic (tumor promotion) differences among mice strains provide evidence for the differences in sensitive and resistant mouse strains. It follows logically that if these differences are associated with susceptibility among mouse strains, then it is reasonable that pharmacokinetic and pharmacodynamic differences between species would also control sensitivity. For example, ODC induction in the guinea pig skin is prostaglandin-independent and the guinea pig is resistant to TPA promotion.

- For the coal tar-derived mixtures to which an occupational cohort may have been exposed and for which a mouse skin painting study was available, skin cancer incidence was either not increased when compared to the expected values or is increased only in association with other risk factors, such as exposure to sunlight.

- In particular, in contrast to the mouse data, the body of evidence clearly indicates that the use of pharmaceutical grade coal tar in the treatment of psoriasis and other skin conditions does not result in an increased incidence of skin cancer in persons exposed to exceedingly high amounts of coal tar for years.

- The data provide clear and convincing evidence that the pharmacokinetic and pharmacodynamic differences between mouse and human skin and can be summarized as follows.

- Anatomic differences, such as the much thinner murine skin, may contribute to the greater penetration and absorption of these compounds in mouse skin than in human skin (Reifenrath et al. 1984).

- The pharmacokinetic and metabolism studies suggest that there are significant quantitative differences in the metabolism of PAHs to their active components (Berry et al. 1977; Das et al. 1986a, 1986b; DiGiovanni 1989; Hall and Grover 1988; Storm et al. 1990).

- Mouse epithelial cells undergo neoplastic transformation when incubated with BaP *in vitro* (Sala et al. 1987). In contrast, human cells have not been transformed in cell culture when incubated with BaP (Fox et al. 1975; Kuroki et al. 1989). Human epidermal cells are capable of being transformed when an initiating dose of a UV-B irradiation or carcinogen (MNNG) is added to a cell system that has been induced to express ODC when immortalized by viruses (Banks-Schlegel and Howley 1983; Rhim et al. 1986).

- Human skin xenografts did not form tumors in response to DMBA alone or with TPA promotion, but did develop benign and malignant tumors when exposed to UV-B light alone or to a similar extent with DMBA and UV-B light (Atillasoy et al. 1997; Graem 1986; Soballe et al. 1996; Urano et al. 1995). Tumors of mouse skin origin formed with all treatment regimens with the exception of studies conducted in the RAG-1 mouse that does not have the DNA repair defect present in the skin of other strains of mice (Atillasoy et al. 1997).

- While the profile of DNA adduct production is less complex in humans than in mice, i.e., (Carmichael et al. 1991; Phillips et al. 1990), DNA adduct formation is found in human skin (Phillips et al. 1990). However, mice

demonstrate much less repair of nontranscribed DNA injured by carcinogens than do humans (Bohr et al. 1985). Coupled with the faster cell turnover rate, fixation of initiated cells by promotional agents is likely to be greater in mouse skin than in human skin.

- Induction of ODC activity, is an obligatory precursor step in mouse skin tumor promotion, which is mediated by-products of COX activity (Verma et al. 1977; Yamamoto et al. 1992). In contrast, the induction of ODC in human epidermis was independent of lipoxygenase and COX pathways (Arnold et al. 1992). It is questionable that the promotional mechanisms operative in murine skin are also operative in human skin (Arnold et al. 1990, 1992; Ashendel and Boutwell 1979; Boutwell 1978; Cameron et al. 1992; Chida and Kuroki 1984; Fischer et al. 1993; Kennard et al. 1995; Lowe 1981; Lowe et al. 1982; Suda et al. 2000; Verma et al. 1977, 1988; Young et al. 1999).

- In conclusion, use of the mouse skin painting studies for quantitative estimates of human skin cancer risk for coal tar-containing shampoos is not appropriate; use of the epidemiological data to assess human skin cancer risk is the only appropriate choice when evaluating coal tar-containing shampoos. However, for comparative purposes only, a quantitative estimate using mouse skin painting data and the appropriate pharmacokinetic adjustments was conducted and is included in Appendix E of this report.

Table 2.1-1
Composition of CTP1 and CTP2

PAH	CTP1 Concentration (µg/g)	CTP1 BaP Equivalent	CTP2 Concentration (µg/g)	CTP2 BaP Equivalent
Anthanthrene	14	0	10.9	0
Anthracene	3454.3	0	4885.7	0
Benz(a)anthracene	26.1	2.6	1223.7	122.4
Benzo(a)pyrene	10.4	10.4	280.9	280.9
Benzo(b)fluoranthene	21.8	2.2	382.3	38.2
Benzo(e)pyrene	8.1	0	248.9	0
Benzo(g,h,i)perylene	3.7	0.4	106.9	10.7
Benzo(g,h,i)fluoranthene	4.9	0	158	0
Benzo[b]naphthol(2,1-d)thiophene	2.8	0	305.7	0
Chrysene	25.6	0.3	1125.4	11.3
Coronene	1.3	0	14	0
Cyclopentapyrene	1.1	0	40	0
Dibenz(a,h)anthracene	0.9	0.3	25.4	8.9
Fluorenthene	3857.14	0	42114.3	0
Indeno(1,2,3-cd)pyrene	4.1	0.4	56.9	5.7
Naphthalene	126,000.0	0	25371.4	0
Perylene	3.7	0	44	0
Phenanthrene	32354.3	0	126285.7	0
Pyrene	1242.9	0	23771.4	0
TOTAL	41037.0	16.6	226451.5	478.0

Table 2.1-2
Incidence of Skin Tumors - CTP1

Lesions	Toluene†	Toluene‡	B(a)P	0.3 mg	1 mg	3 mg	9 mg
No. of Animals:	62	62	62	62	62	62	62
Squamous-cell carcinoma	0 (0%)	0 (0%)	30*** (48%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)
Papilloma	0 (0%)	0 (0%)	27*** (44%)	0 (0%)	0 (0%)	0 (0%)	2 (3%)
Basal-cell carcinoma	0 (0%)	0 (%)	4 (6%)	0 (%)	0 (0%)	0 (0%)	0 (0%)
Basal cell tumor, benign	0 (0%)	0 (%)	1 (2%)	0 (%)	0 (0%)	0 (0%)	0 (0%)
Sebaceous carcinoma	0 (0%)	0 (%)	1 (2%)	0 (%)	0 (0%)	0 (0%)	0 (0%)
Sebaceous adenoma	0 (0%)	0 (%)	2 (3%)	0 (%)	0 (0%)	0 (0%)	0 (0%)
Malignant fibrous histiocytoma	0 (0%)	0 (%)	1 (2%)	0 (%)	0 (0%)	0 (0%)	0 (0%)
Cavernous haemangioma	1 (2%)	0 (%)	0 (0%)	0 (%)	0 (0%)	0 (0%)	0 (0%)
Total number of animals with skin tumors	1	0	47	0	0	1	2

Source: Fraunhoffer (1997).

† Represents control group for the 1, 3, and 9 mg treatment groups.
‡Represents control group for the 0.3 mg treatment group.

Significance of difference in a pairwise Fisher's test between control and treatment groups:
*P<0.05, **P<0.01, ***P<0.001

Table 2.1-3
Incidence of Selected Nonneoplastic Skin Lesions - CTP1

Lesions	Toluene†	Toluene‡	B(a)P	0.3 mg	1 mg	3 mg	9 mg
No. of Animals:	62	62	62	62	62	62	61
Epidermal hyperplasia with cellular atypia							
Moderate	0 (0%)	0 (%)	3 (5%)	0 (%)	0 (0%)	0 (0%)	0 (0%)
Severe	0 (0%)	1 (2%)	26*** (42%)	0 (0%)	2 (2%)	1 (2%)	2 (3%)
Score expanded totals	0 (0%)	1 (2%)	29*** (47%)	0 (0%)	2 (3%)	1 (2%)	2 (3%)
Epidermal hyperplasia							
Very slight	2 (3%)	0 (%)	0 (0%)	0 (%)	2 (3%)	0 (0%)	0 (0%)
Slight	28 (45%)	22 (35%)	3*** (5%)	30 (48%)	22 (35%)	26 (42%)	20 (32%)
Moderate	28 (45)	29 (47%)	21 (34%)	23 (37%)	26 (42%)	23 (37%)	25 (40%)
Severe	4 (6%)	10 (16%)	9 (15%)	9 (15%)	10 (16%)	12 (19%)	15* (24%)
Score expanded totals	62 (100%)	61 (98%)	33*** (53%)	62 (100%)	60 (97%)	61 (98%)	60 (97%)
(Superficial) purulent dermatitis							
Slight	22 (35%)	24 (39%)	7** (11%)	29 (47%)	22 (35%)	29 (47%)	24 (39%)
Moderate	2 (3%)	3 (5%)	2 (3%)	2 (3%)	2 (3%)	1 (2%)	2 (3%)
Severe	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)
Score expanded totals	25 (40%)	27 (44%)	9** (15%)	31 (50%)	24 (39%)	30 (48%)	27 (44%)
Hyperkeratosis							
Very slight	0 (0%)	0 (%)	0 (0%)	0 (%)	1 (2%)	0 (0%)	0 (0%)
Slight	39 (63%)	23 (37)	6*** (10%)	33 (53%)	32 (52%)	26* (42%)	16*** (26%)
Moderate	22 (35%)	34 (55%)	36* (58%)	25 (40%)	21 (34%)	31 (50%)	35* (56%

Table 2.1-3 (continued)

Lesions	Toluene†	Toluene‡	B(a)P	0.3 mg	1 mg	3 mg	9 mg
No. of Animals:	62	62	62	62	62	62	61
Severe	0 (0%)	5 (8%)	19*** (31%)	4 (6%)	8** (13%)	5 (8%)	11*** (18%)
Score expanded totals	61 (98%)	62 (100%)	61 (98%)	62 (100%)	62 (100%)	62 (100%)	62 (100%)
Ulcerative dermatitis (ulceration)							
Slight	16 (26%)	14 (23%)	2*** (3%)	8 (13%)	3** (5%)	6* (10%)	7 (11%)
Moderate	5 (8%)	8 (13%)	4 (6%)	8 (13%)	6 (10%)	6 (10%)	7 (11%)
Severe	1 (2%)	10 (16%)	13** (21%)	7 (11%)	12** (19%)	16*** (26%)	17*** (27%)
Score expanded totals	22 (35%)	32 (52%)	19 (31%)	23 (37%)	21 (34%)	28 (45%)	31 (50%)

Source: Fraunhoffer (1997).

† Represents control group for the 1, 3, and 9 mg treatment groups.
‡ Represents control group for the 0.3 mg treatment group.

Significance of difference in a pairwise Fisher's test between control and treatment groups:
*P<0.05, **P<0.01, ***P<0.001

Table 2.1-4
Incidence of Skin Tumors - CTP2

Lesions	Toluene†	Toluene‡	B(a)P	0.1 mg	0.3 mg	1 mg	3 mg	9 mg
No. of Animals	62	62	62	62	62	62	62	61
Squamous-cell carcinoma	0 (0%)	0 (0%)	30*** (48%)	1 (2%)	1 (2%)	3 (5%)	16*** (26%)	6* (10%)
Papilloma	0 (0%)	0 (0%)	27*** (44%)	0 (0%)	2 (3%)	6* (10%)	12*** (19%)	15*** (24%)
Basal-cell carcinoma	0 (0%)	0 (0%)	4 (6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Basal cell tumor, benign	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Sebaceous carcinoma	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Sebaceous adenoma	0 (0%)	0 (0%)	2 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Malignant fibrous histiocytoma	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cavernous haemangioma	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)
Total number of animals with skin tumors	1	0	47	1	3	9	23	20

Source: Fraunhoffer (1997).

† Represents control group for the 1, 3, and 9 mg treatment groups.
‡ Represents control group for the 0.1 and 0.3 mg treatment groups.

Significance of difference in a pairwise Fisher's test between control and treatment groups:
*P<0.05, **P<0.01, ***P<0.001

Table 2.1-5
Incidence of Nonneoplastic Skin Lesions - CTP2

Lesions	Toluene†	Toluene‡	B(a)P	0.1 mg	0.3 mg	1 mg	3 mg	9 mg
No. of Animals	62	62	62	62	62	62	62	61
Epidermal hyperplasia with cellular atypia								
Moderate	0 (0%)	0 (%)	3 (5%)	0 (%)	0 (%)	0 (0%)	1 (2%)	0 (0%)
Severe	0 (0%)	1 (2%)	26*** (42%)	0 (0%)	0 (0%)	5 (8%)	10** (16%)	13*** (21%)
Score expanded totals	0 (0%)	1 (2%)	29*** (47%)	0 (0%)	0 (0%)	5 (8%)	11*** (18%)	13*** (21%)
Epidermal hyperplasia								
Very slight	2 (3%)	0 (%)	0 (0%)	0 (%)	0 (%)	1 (2%)	1 (2%)	0 (0%)
Slight	28 (45%)	22 (35%)	3*** (5%)	42*** (68%)	37** (60%)	20 (32%)	7*** (11%)	6*** (10%)
Moderate	28 (45%)	29 (47%)	21 (34%)	14** (23%)	20 (32%)	25 (40%)	22 (35%)	22 (35%)
Severe	4 (6%)	10 (16%)	9 (15%)	5 (8%)	4 (6%)	10 (16%)	9*** (31%)	20*** (32%)
Score expanded totals	62 (100%)	61 (98%)	33*** (53%)	61 (98%)	61 (98%)	56* (90%)	49*** (79%)	49*** (79%)
(Superficial) purulent dermatitis								
Slight	22 (35%)	24 (39%)	7** (11%)	33 (53%)	30 (48%)	26 (42%)	6*** (10%)	16 (26%)
Moderate	2 (3%)	3 (5%)	2 (3%)	3 (5%)	0 (0%)	3 (5%)	5 (8%)	6 (10%)
Severe	1 (2%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Score expanded totals	25 (40%)	27 (44%)	9** (15%)	37 (60%)	30 (48%)	29 (47%)	11* (18%)	23 (37%)
Hyperkeratosis								
Very slight	0 (0%)	0 (%)	0 (0%)	0 (%)	0 (%)	0 (0%)	0 (0%)	0 (0%)
Slight	39 (63%)	23 (37%)	6*** (10%)	44*** (71%)	44*** (71%)	23** (37%)	12*** (19%)	9*** (15%)
Moderate	22 (35%)	34 (55%)	36* (58%)	15*** (24%)	17** (27%)	29 (47%)	30 (48%)	36* (58%)

Table 2.1-5 (continued)

Lesions	Toluene†	Toluene‡	B(a)P	0.1 mg	0.3 mg	1 mg	3 mg	9 mg
No. of Animals	62	62	62	62	62	62	62	61
Severe	0 (0%)	5 (8%)	19*** (31%)	3 (5%)	1 (2%)	9** (15%)	18*** (29%)	15*** (24%)
Score expanded totals	61 (98%)	62 (100%)	61 (98%)	62 (100%)	62 (100%)	61 (98%)	60 (97%)	61 (98%)
Ulcerative dermatitis (ulceration)								
Slight	16 (26%)	14 (23%)	2*** (3%)	4* (6%)	5* (8%)	2*** (3%)	6* (10%)	7 (11%)
Moderate	5 (8%)	8 (13%)	4 (6%)	2 (3%)	6 (10%)	6 (10%)	3 (5%)	6 (10%)
Severe	1 (2%)	10 (16%)	13** (21%)	5 (8%)	6 (10%)	13** (21%)	31*** (50%)	22*** (35%)
Score expanded totals	22 (35%)	32 (52%)	19 (31%)	11*** (18%)	17* (27%)	21 (34%)	40** (65%)	35* (56%)

Source: Fraunhoffer (1997).

† Represents control group for the 1, 3, and 9 mg treatment groups.
‡ Represents control group for the 0.1 and 0.3 mg treatment groups.

Significance of difference in a pairwise Fisher's test between control and treatment groups:
*P<0.05, **P<0.01, ***P<0.001

Table 2.1-6
Skin Tumor Incidence Reported by Warshawsky et al. (1993)

Treatments (Dose)	Incidence of Tumors (Latency Period, weeks)	% Tumor Incidence
BaP (0.0006%) in toluene	0/66	0
Coal tar in toluene	39/76 (73)	51
BaP (0.0006%) in toluene + 50 µl –dodecane	0/10 (0)	0
Coal tar in toluene + 50 µl –dodecane	17/26 (39)	65
BaP (0.001%)	0/14	0
Anthracene (0.1%)	0/14	0
Anthracene (0.1%) + BaP (0.001%)	1/13 (85)	8
Chrysene (0.1%)	1/15 (81)	7
Chrysene (0.1%) + BaP (0.001%)	3/13 (73)	23
Fluoranthene (0.1%)	0/15	0
Fluoranthene (0.1%) + BaP (0.001%)	1/12 (95)	8
Phenanthrene (0.1%)	1/12 (100)	8
Phenanthrene (0.1%) + BaP (0.001%)	1/17 (53)	6
Pyrene (0.1%)	1/13 (96)	8
Pyrene (0.1%) + BaP (0.001%)	0/13	0
Anthracene (0.1%) + Chrysene (0.1%) + Fluoranthene (0.1%) + Phenanthrene (0.1%) + Pyrene (0.1%)	3/13 (73)	23
Anthracene (0.1%) + Chrysene (0.1%) + Fluoranthene (0.1%) + Phenanthrene (0.1%) + Pyrene (0.1%) + BaP (0.001%)	8/17 (56)	47
High temperature coke oven tar	39/76 (73)	51

Table 2.1-7
The Effects of Solvents on the Carcinogenic Potential of Benz(a)anthracene (BA) Compounds

Chemical	Solvent	% of Mice with Tumors	Average Latent Period (Weeks)
no treatment		–	–
toluene	toluene	–	–
n-dodecane	n-dodecane	0	–
0.2% 9-methyl BA	toluene	10	90
0.2% 9-methyl BA	n-dodecane	76	38
0.2% 2-methyl BA	Toluene	--	--
0.2% 2-methyl BA	n-dodecane	44	66
0.2% 3-methyl BA	toluene	--	--
0.2% 3-methyl BA	n-dodecane	88	77
0.2% 8-methyl BA	toluene	94	41
0.2% 8-methyl BA	n-dodecane	100	25
0.2% dibenz(a,h)anthracene	toluene	95	35
0.2% dibenz(a,h)anthracene	30% toluene 70% n-dodecane	100	26

Table 2.1-8 Summary of Animal Studies

Reference	Protocol	Conclusions
Fraunhofer 1997	Two different coal tar creosote mixtures (100% coal tar distillate), designated as CTP1 (low BaP content) and CTP2 (high BaP content), were applied to CD-1 mice in amounts of 0 (toluene vehicle control), 0.1 (CTP2 only), 0.3, 1, 3 or 9 mg (25 Fl/treatment) twice a week for 78 weeks. An additional group of 62 mice received 7.5 Fg of the positive control, BaP.	The data reported in this study suggest that the nonneoplastic effects, particularly tissue damage (ulceration) and hyperplasia, were associated with the inflammatory/irritating properties of these mixtures and may have contributed to the carcinogenic activity of the PAH mixture. Although ulceration and hyperplasia were observed with both coal tar mixtures (CTP1 and CTP2), the severity of these lesions was greatest with CTP2, the mixture where increases in skin cancer incidence were observed following application. Further, both the number of animals diagnosed with one or more of these noncancer lesions as well as the severity of the lesions increased in a dose-dependent manner. Therefore, it is possible that the potential for the coal tar mixture to induce tissue damage, with subsequent regeneration and repair, could be related to the carcinogenic potency of the mixture.
Warshawsky et al. 1993	The five individual PAHs test included anthracene, chrysene, fluoranthene, phenanthracene, pyrene, and BaP or a high temperature coke oven tar containing BaP. In the first set of experiments, 0, 0.1% toluene solutions of each of the five PAHs listed above, 0.1% toluene solutions of each of the five PAHs that also contained 0.001% BaP, a toluene solution containing all five PAHs at 0.1% and no BaP, or a toluene solution containing all five PAHs at 0.1%, plus 0.001% BaP were applied in a 50 µl volume to the shaved, occluded skin of C3H/HeJ mice (20 animals/group) twice weekly for 2 years. In a second set of experiments, mice (20 animals/group) were dermally exposed twice weekly for 2 years with 0, 50 µl of 0.2% of a series of BA derivatives, including 9-methyl-, 2-methyl, 3-methyl-, 8-methyl-, and dibenzanthracene, in toluene, dodecane, or 30% toluene and 70% n-dodecane. In the third set of experiments, 0, 50 µl volumes of 0.0006% BaP in toluene, coal tar in toluene such that it contained 0.0006% BaP, 0.0006% BaP in toluene followed by 50 µl of n-dodecane, or coal tar in toluene, such that it contained 0.0006% BaP, followed by 50 µl n-dodecane, were applied twice weekly to the skin of mice (20 animals/group).	The authors noted that "the interactions of individual PAHs with BaP vary depending on the PAHs studied," and suggested that the differences in carcinogenic potential between mixtures were likely due to specific interactions between the PAHs at the level of metabolism, DNA adduct formation, or DNA adduct repair. Warshawsky et al. (1993) concluded that the potency of a PAH mixture could not be predicted by the BaP content alone.
Wright et al. 1985	Charles River CD-1 mice received a single 25 mg application of the National Bureau of Standards (NBS) coal tar or the coal tar portion of a 20% pharmaceutical stock material in methylene chloride applied in a 50 µl volume to a shaved area of the back. A 50 µl volume of phorbol myristate acetate was applied twice weekly for 6 months, beginning 2 weeks after initiation. An additional group of animals received a topical application of methylene chloride only, and served as vehicle controls.	At 57 days post-initiation, tumor incidences were approximately 15% and 30% in the pharmaceutical stock and NBS groups, respectively. A tumor incidence of 100% was reported at 169 days post-initiation for both test materials. However, the cumulative number of tumors normalized for 30 mice/group was greater in the NBS coal tar group than in the pharmaceutical stock group, approximately 180 and 140, respectively, at 169 days. The total PAH content in the NBS coal tar and in the 20% stock material were 643.18 and 565.03 parts per thousand, respectively, approximately a 12% difference in PAH content between test materials. However, the BaP content in the 20% stock solution was approximately 69% less than the BaP content in the NBS coal tar. Carcinogenic potential could not be predicted from BaP or BaP-equivalents alone.

Table 2.1-8 (continued)

Reference	Protocol	Conclusions
Nesnow et al. 1982	Roofing tar and coke oven particulate emission samples were applied topically to 7- to 9-week-old SENCAR mice (40/sex/group) at quantities of 0.10, 0.50, 1.0, 2.0, or 10.0 mg. BaP was applied in at quantities of 0.00252, 0.01262, 0.05046, or 0.10092 mg. One group of mice was topically treated with 0.2 ml of acetone only and served as the vehicle controls. One week following treatment, all mice were topically administered 2.0 µg of TPA in 0.2 mL acetone, twice weekly.	When comparing the incidence of papillomas in the BaP treatment groups versus the incidence of papillomas in the roofing tar and coke oven treatment groups that had similar BaP contents, the incidence of tumors varied, even if BaP contents were similar. For example, BaP applied at a quantity of 0.00252 mg resulted in male and female mice with a 45% or 31% incidence of papillomas, respectively. However, the 2 mg coke oven sample, with similar BaP content (4.78E-3mg BaP), resulted in a much larger incidence of papillomas for male (95%) and female (90%) mice. Also, similar BaP contents in both roofing tar and coke oven samples resulted in very different tumor incidences. For example, 2 mg of roofing tar or coke oven sample had similar BaP content (1.778E-3 or 9.56E-4 mg, respectively), but produced distinctly different incidences of papillomas (36% males, 37% females or 95% males, 90% females, respectively). The results of this study suggest that even with similar BaP content in the test material, differing tumor incidence is observed.
Grimmer et al. 1984	Grimmer et al. (1984) conducted a study to identify components of and determine the potential carcinogenic impact of emissions from coal-fired residential furnaces using CFLP mice. Various fractions of the emissions were evaluated for carcinogenicity by comparing the fractions to the proportion to total condensate (wt%).	Results of Probit and Weibull analyses indicated that the PAHs and thiaarenes with more than three aromatic rings contained in coal tar residue are the major contributors (109-118% compared with the total condensate) to the observed carcinogenicity of the coal tar residue. BaP contributed 10-11% of the total carcinogenicity even though only a small portion of the total condensate contained BaP (0.702 mg/g condensate). However, the polyaromatic compounds (PAC)-free fraction, the fraction containing PACs with 2 and 3 rings were reported with no activity. Similar potency results between flue gas condensate (100 or 100%) and PAH-fraction with more than 3 rings (118 or 109%) were reported by Probit and Weibull analysis, respectively, with flue gas condensate (100 wt%) contributing a greater proportion to the total condensate, when compared to the PAH-fraction with more than 3 rings (15.2 wt%).
NIOSH 1979	NIOSH (1979) conducted a study to determine the biologic activity of 16 coal tar neutral subfractions of a composite coal tar material through the use of a mouse-skin bioassay utilizing C3H/HeJ male mice. However, due to the expiration of the project, only seven subfractions were able to be tested. Twice a week, a 100 µl volume of solution was applied to the shaven skin of male mice (15/group), at least 3 days apart, for a total of 26 weeks. Each 100 µl solution contained 5 mg of the test material.	Squamous cell papillomas were observed in 2, 6 and 1 animals from Subfraction groups 7 (possibly pyrene, phenanthrene, and substituted 3-ring compounds), 9 (mostly condensed 4-ring systems, with possibly BA and/or chrysene as major components), and 13 (possibly polar polynuclear compounds), respectively. SCCs were reported in five and six animals from Subfraction groups 9 or 10 (probably 5-ring systems and higher, possibly BaP), respectively. There were no tumors reported for animals in the control group, or animals in any other subfraction group. Overall, this study showed that the 4- and 5-ring PAHs produced the greatest biologic activity, with no carcinogenic tumors produced by PAHs with 3-rings or less.

Table 2.2-1
Summary of Occupational Non-Melanoma Skin Cancer (a)

Study	Cases	Comments
Exposure to Soot		
Henry 1937	103	Chimney sweep was listed as the occupation for 103 deaths out of a total of 1,487 deaths resulting from scrotal cancer between the years 1911 and 1935 based on records kept by the Register General for England and Wales.
Waldron et al. 1984	7	The study evaluated the Cancer Registry entries made between 1936 and 1976 for the West Midland Area in England. The follow-up period was at minimum 5 years. The incidence of scrotal cancer in sweeps represented only 2% of the 344 cases of scrotal cancer identified in the study. Scrotal cancer incidence in individuals with no known exposure to PAHs represented 16.6% of the total cases.
Evanoff et al. 1993	4	A case control study on 5,542 chimney sweeps employed from 1958-1987 in Sweden found only 4 cases of nonmelanoma skin cancer as opposed to an expected value of 6.5.
Exposure to Coal Tar and Pitch Workers in the paraffin, tar distillation, coke production, coke oven workers, hydrogenation of coal, gas-works, aluminum plant workers, carbon, patent fuel, asphalt, and roofing industries		
Volkman 1875 [1]	3	Case reports on individuals employed in the paraffin industry
Bell 1876 [1]	2	Case reports on individuals employed in the paraffin industry
Ball 1885 [1]	2	Case reports on individuals employed at a coal tar distillery
Lueke 1907	3	Case reports on individuals employed in the carbon industry
Ross 1948	358 91	The number of cases of skin cancer in individuals employed at coal tar distilleries reported to the Chief Inspector of Factories in Britain. The number of cases of skin cancer in individuals employed in the gas-works industry reported to the Chief Inspector of Factories in Britain.
Hoffman 1928	7 11	Seven deaths from scrotal cancer were reported in a population of 1,150 patent fuel workers in England and Wales between 1911 and 1920. Data was taken from death certificates and census data for 1911. Eleven deaths from scrotal cancer were reported in a population of 54,447 gas-works employees in England and Wales between 1911 and 1920. Data was taken from death certificates and census data for 1911.
Henry 1947 [2]	2,229	Of 3,753 total skin cancer cases reported to the Chief Inspector of Factories in Britain, 2,229 were thought to be the result of exposure to coal tar or pitch; however, no attempts were made to illustrate numbers by occupation.

Table 2.2-1 (continued)

Study	Cases	Comments
Heller 1930		All identified cases of skin cancer at the following industries and hospitals between 1920 and 1928 based on interviews and records.
	0	No skin cancer was reported in a cohort of 300 workers employed at ten coal tar distillation facilities operated by the American Tar Products Company.
	0	No skin cancer was reported in workers employed at coal tar distillation facilities operated by the Chicago Gas Company
	0	No skin cancer was reported in workers employed at coal tar distillation facilities or coke ovens operated by the Chicago By-Products Company
	0	No skin cancer was reported in workers employed at various coal tar distillation facilities primarily producing pitch in Chicago.
	19	Nineteen cases of skin cancer treated at Memorial Hospital, New York, and the New York Skin and Cancer Hospital between 1920 and 1928 were reported to be the result of exposure to coal tar or pitch; four - roofers, two - coal tar distillery workers, one - coke oven worker, 12 - gas works employees.
	21	Three scrotal tumors, seven tumors on the head or neck, and 11 tumors on the hand, wrist or arm were reported in an unspecified number of workers employed at a Cleveland Industrial Plant that used large quantities of pitch.
Wood 1929 [3]	0	No cases of skin cancer were reported for 88 coal tar handlers at various gas-works or coke oven facilities in Pennsylvania.
	0	No cases of skin cancer were reported for 750 workers employed by the Philadelphia Gas Company.
	0	No cases of skin cancer were reported for 500 workers employed at the Barret distillation plant.
Schamberg 1910 [3]	5	Five cases of skin cancer, one located on the scrotum and four located on the wrist or hand, were reported in workers exposed to coal tar for between 13-55 years at a roofing paper manufacturing plant. The scrotal cancer was preceded by preliminary skin irritation.
Sexton 1960	5	Four cases of SCC located on the head, hand, or buttocks, and one case of BCC were reported in a cohort of 359 workers employed at a coal hydrogenation plant. Latency period ranged between 9 and 116 months. The authors reported that incidence rate was significantly increased when compared to the background incidence rate of skin cancer reported by Dorn and Cutler.
Waldron et al. 1984	27	The study evaluated the Cancer Registry entries for the West Midlands Area in England made between 1936 and 1976. The follow-up period was at minimum 5 years. The incidence of scrotal cancer in individuals exposed to tar or pitch represented only 7.9% of the 344 cases of scrotal cancer identified in the study. Scrotal cancer incidence in individuals with no known exposure to PAHs represented 16.6% of the total cases.

Table 2.2-1 (continued)

Study	Cases	Comments
Hammond et al. 1976	5	Reported on the deaths from skin cancer in roofers belonging to the United Slate, Tile and Composition Roofers, Damp and Waterproof Workers Association Union. In union members of 9-19 years, two incidence of cancer were observed as opposed to 0.43 expected (SMR = 4.65). In union members of 20 + years, three cases of skin cancer were noted as opposed to 0.75 expected (SMR = 4.00). Exposure data was presented, and ranged between 14 µg BaP/m3 in the roof tarring area to 6,000 µg/m^3 in the coal tar roofing kettle area. However, the cases of skin cancer were not identified by job category.
Letzel and Drexler 1998	249	The cohort consisted of individuals recognized as having occupationally induced disease between 1946 and 1996. The authors noted that chronic irritation and inflammation was an obligatory precursor to skin cancer development. Although the authors suggest that the role of UV light in skin cancer was overvalued over 90% of the SCCs and BCCs were located in sun exposed areas. Thus, there is a high probability that UV light did contribute to the formation of tumors in this cohort.
Sherson et al. 1991	65	The standard morbidity ratio (SMR) for nonmelanoma skin cancer was 0.93 (65 cases observed/69.86 cases expected), and thus, the authors reported that there was no significant increase in the incidence of skin cancer in this cohort of foundry workers, when compared to the general population.
Spinelli et al. 1991	38	The SIR for non-melanoma skin cancer was only 0.51 (38 cases observed/74.31 cases expected = 0.51) in this cohort of aluminum workers exposed to coal tar pitch volatiles.
Rønneberg and Andersen 1995	8	The Standardized Incidence Ratio (SIR = observed/expected) for skin cancer in workers employed for less than 3 years was 3.09. However, for workers employed for 3 or more years, the SIR for skin cancer was only 0.79.
Exposure to Creosote		
Karlehagen et al. 1992	9	A marginally statistically significant increase in the incidence of nonmelanoma skin cancer was reported (9 cases observed/3.79 expected; SIR = 2.37). As noted by the authors, comparisons were made to national cancer rates, which could have introduced bias into the study because the base risk of cancer differed between urban and rural areas where the plants were located, and the workers worked partly outdoors and were exposed to UV light, which likely contributed to the incidence of skin cancer.
Exposure to mineral and cutting oils.		
Hoffman 1928	100	Number of deaths occurring in a cohort of 74,625 mule spinners between 1911 and 1920 based on death certificates and census data for England and Wales.
Henry 1937	449	Number of deaths occurring in mule spinners reported between 1911 and 1935. Approximately 76% of the cancer cases involved the scrotum.

Table 2.2-1 (continued)

Study	Cases	Comments
Heller 1930	5	A total of five deaths from skin cancer were reported in mule spinners for the areas of Fall River and New Bedford in the United States.
	6	Six cases of scrotal cancer out of a total of 25 cases treated from 1887-1928 at Massachusetts General Hospital, Huntington Memorial Hospital, and City Hospital, Boston
Waldron et al. 1984	213	The study evaluated the Cancer Registry entries for the West Midland Area in England made between 1936 and 1976. The follow-up period was at minimum 5 years. The incidence of scrotal cancer in individuals exposed to mineral oils, including the cotton mule spinners, represented 61.9%% of the 344 cases of scrotal cancer identified in the study. Scrotal cancer incidence in individuals with no known exposure to PAHs represented 16.6% of the total cases.

[a] All skin cancers were nonmelanoma unless otherwise stated in the comments for each study.
[1] Data was extracted from the review on scrotal cancer presented by Butlin (1892).
[2] Data was extracted from IARC (1984).
[3] Data was extracted from the review on skin cancer presented by Heller (1930).

Table 2.2-2: Summary Table of Epidemiology Studies Conducted in Patients with Psoriasis and Atopic Dermatitis

Study	Population Description and Control Group	Primary Treatments	Number of Individuals	Average Years of Follow up	Skin Cancer Incidence	Comments
Stern and Laird 1994	Psoriasis NCS [1] (control)	Mixed NA	1,380	13.2	O: SCC - 326 O: BCC - 217 E: NR	The authors reported that no increase in the incidence of skin cancer was associated with the use of tar and UV-B; however, an increase in the incidence of skin cancer was associated with PUVA and methotrexate
Jones et al. 1985	Psoriasis WSCR [2] (control)	Coal Tar NA	719	10	O: 3 E: 1.6	No increase in the incidence of skin cancer was reported.
Pittelkow et al. 1981	Psoriasis TNCS [3] (control)	Coal Tar and UV-B light	280	20.1	O: 19 E: 26.6	The authors reported no increase in skin cancer when compared to the expected numbers.
Grupper and Berretti 1980	Psoriasis	Coal Tar and UV-B light	1,000 4,000	NR NR	NR NR	No increase in the incidence of skin cancer was apparent in either of these groups
Menter and Cram 1983	Psoriasis	Coal Tar and UV-B light	300	> 1 yr	NR	No increase in the incidence of skin cancer was noted.
Maughan et al. 1980	Atopic Dermatitis TNCS [3] (control)	Coal Tar and UV-B light	426 NA	25 NA	O: 11 E: 10	The authors reported no increase in skin cancer when compared to the expected numbers.
Jemec and Østerlind 1994	Atopic Dermatitis	Coal Tar	117	37.2	0	No increase in skin cancer was observed.
Bhate et al. 1993	Psoriasis Normal (control)	Mixed None	2,247 4,494	NR NA	O: 34 O: 44	There was no significant increase in skin cancer in the cohort overall. A significant increase was established for the female constituency (p = 0.03); however, there was no effect due to tar treatment.
Stern et al. 1985	Psoriasis Normal (control)	Mixed	4,190 7,280	NR NR	NR NR	The authors concluded that the increase in skin cancer in psoriasis patients was at least as great as that observed in the general population
Alderson and Clark 1983	Psoriasis SNCR [4]	Mixed	8,405	11.5	O: 51 E: 43.6	The expected value for this cohort was 43.6 (O/E = 1.17). The authors concluded that there was no significant increase in the incidence of skin cancer when compared to the expected value.
Halprin et al. 1982	Psoriasis Diabetics (control)	Mixed NA	150 150	NR	O: 20 E: 7	The author concluded that there was an increase in the incidence in this cohort of patient.

[1] National Cancer Survey (Scotto et al. 1983); [2] West Scotland Cancer Registry; [3] Third National Cancer Survey (Scotto et al. 1974); [4] Scottish National Cancer Registry
NR = Not Reported; NA = Not Available; O = Observed; E = Expected.

Table 2.3-1
Differences Between Human Skin and Rodent Skin

Parameters	Human	Rodent
Epidermal thickness[1]	Variable mean = 100 Fm	Constant mean = 30 Fm
Epidermal layers (number)[1]	$10	<10
Differences in epidermal layers[1]	Thick granular and keratinized layers	Thin granular and keratinized layers
Epidermal cell turnover time	13-71 days[2,3,4]	6-34 days[5, 6, 7]
Location of epidermal mitosis[1]	Basal and parabasal cells	Basal cells
Hair distribution[1]	Variable	Homogeneous
Sweat glands[1]	Abundant	None or infrequent

1 - Klein-Szanto et al. (1991)
2 - Rice and Cohen (1996)
3 - Epstein and Maibach (1965)
4 - Bloom and Fawcett (1975)
5 - Iverson et al. (1968)
6 - Bertalanffy and Lau (1962)
7 - Bertalanffy (1964)

Table 2.3-2
Aryl Hydrocarbon Hydroxylase Inducibility in Mice and Human Skin

Mouse (pmole/min/mg protein)		Human (pmole/min/mg protein)		Reference
Basal	Induced	Basal	Induced	
Induced with Benz(a)anthracene				
1.1 1.3 1.7	8.5 12.5 12.5			Thompson and Slaga (1976) (1) Thompson and Slaga (1976) (1) Kinoshita and Gelboin (1972) (1)
		0.1 0.1 0.06	0.4 0.33 0.165	Levin et al. (1972 (2) Alvares et al. (1972) (2) Bickers et al. (1984) (2)
Induced with DMBA				
1.2	8.33			Kinoshita and Gelboin (1972) (1)
Induced with Coal Tar				
0.59	16.43	0.45 0.045	2.29 0.165	Das et al. (1986a) (3) Bickers and Kappas (1978) (4)
Uninduced				
3.25		0.24		Storm et al. (1990) (5)

1 BA (100-200 µmoles) applied to mouse skin 8-24 hours before harvesting; BaP (100 nmoles) added to epidermal cell homogenate for 30 minutes. Control mice vehicle only.
2 Human neonatal foreskin or adult epidermal homogenates incubated with or without BA (10-13 µmoles) for 16-24 hours; BaP (100 nmoles) added for 30 minutes.
3 Crude coal tar (0.1 ml) was applied to a human skin xenograft and to distal host mouse skin for 24 hours before harvesting epidermis; BaP (100 nmoles) added for 30 minutes.
4 A 20% coal tar solution was applied to the skin of adult human volunteers for 24 hours under occluded dressing. Biopsy whole skin homogenates from treated and untreated areas incubated with BaP (100 nmoles) for 30 minutes.
5 Application of 3 µg BaP/cm^2 applied for 30 minutes.

Table 2.3-3
Comparative Metabolism of Benzo(a)pyrene in Mouse and Human Skin

Metabolized to:	Mouse Skin % Amount Applied	Human Skin % Amount Applied
Total BaP metabolites	6.0 [1]	<0.05 [1]
Total BaP diol metabolites	1.5 [2]	0.015 [3]
Total BaP 7,8-diol metabolites	1.05 [4]	0.0063 [3]

[1] Storm et al. (1990). Based on application of 3 µg BaP/cm^2 skin in flow through cell system for 24 hours. Human value is at the detection limit of 6 pmole BaP metabolites/cm^2.
[2] Storm et al. (1990). Based on application of 3 µg BaP/cm^2 skin for 24 hours. Total BaP metabolites fractionated to relevant categories, i.e., total diols.
[3] Hall and Grover (1988). Application of 25 µg BaP/cm^2 human skin in flow through cell system. After 20 hours of exposure, total diol was 15 pmoles/cm^2 applied (100 µmoles BaP applied).
[4] Berry et al. (1977) and DiGiovanni (1989). Incubation of skin homogenate with 0.2 nmoles (25 µg/mL) BaP for 60 minutes.

Figure 2-1
Microscopic Anatomy of Human Skin

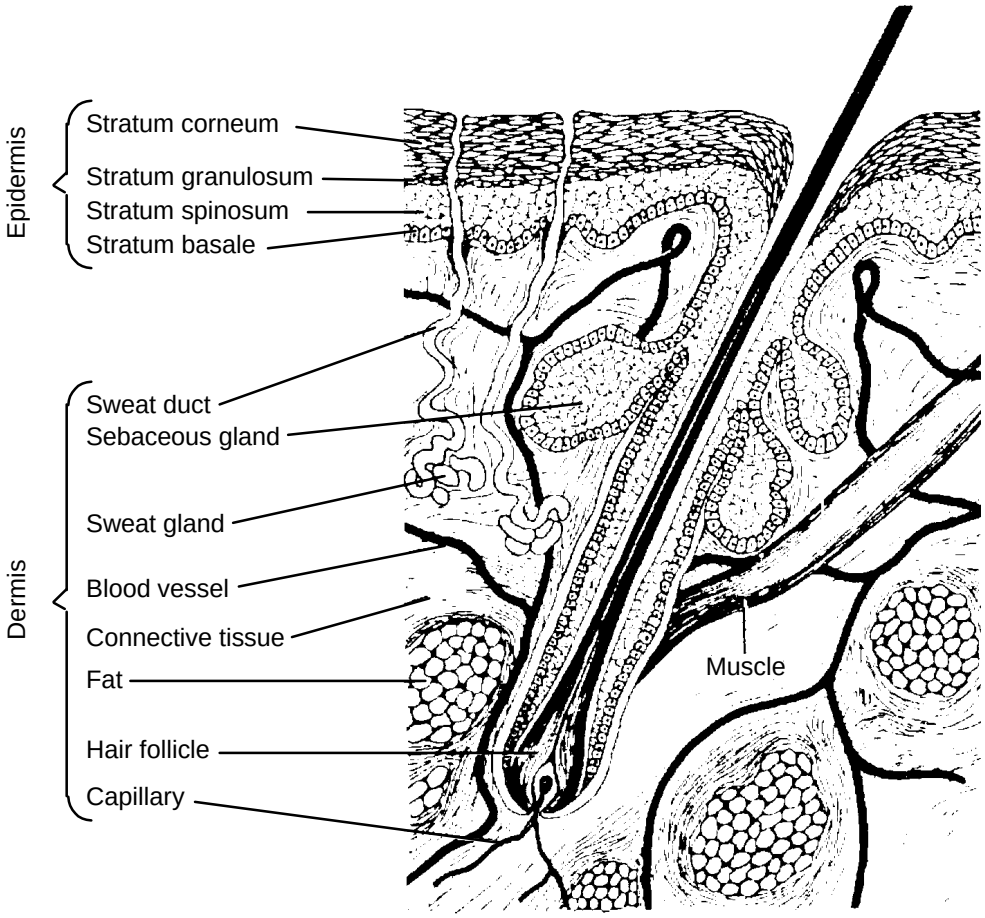


Figure 2-2
Proposed Mode of TPA Induced Promotion in the Mouse

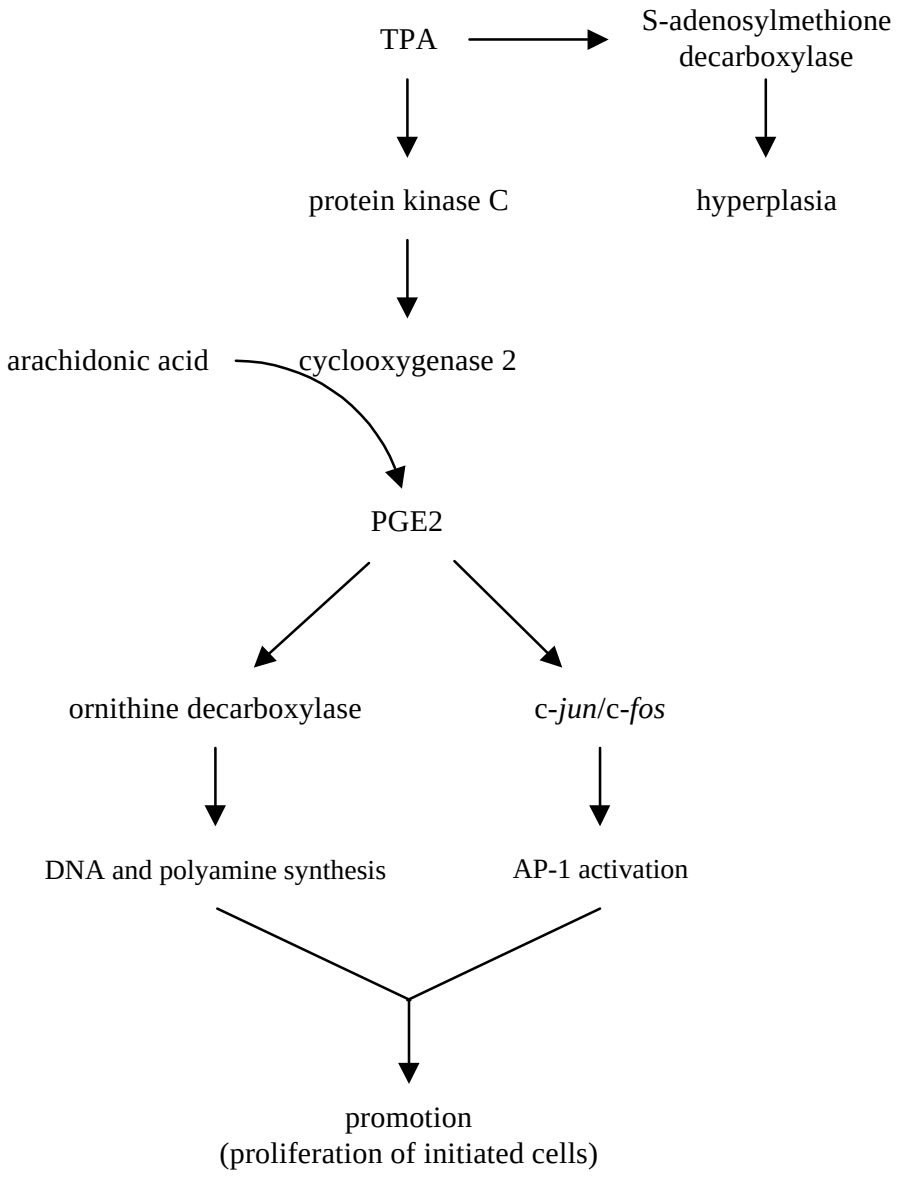
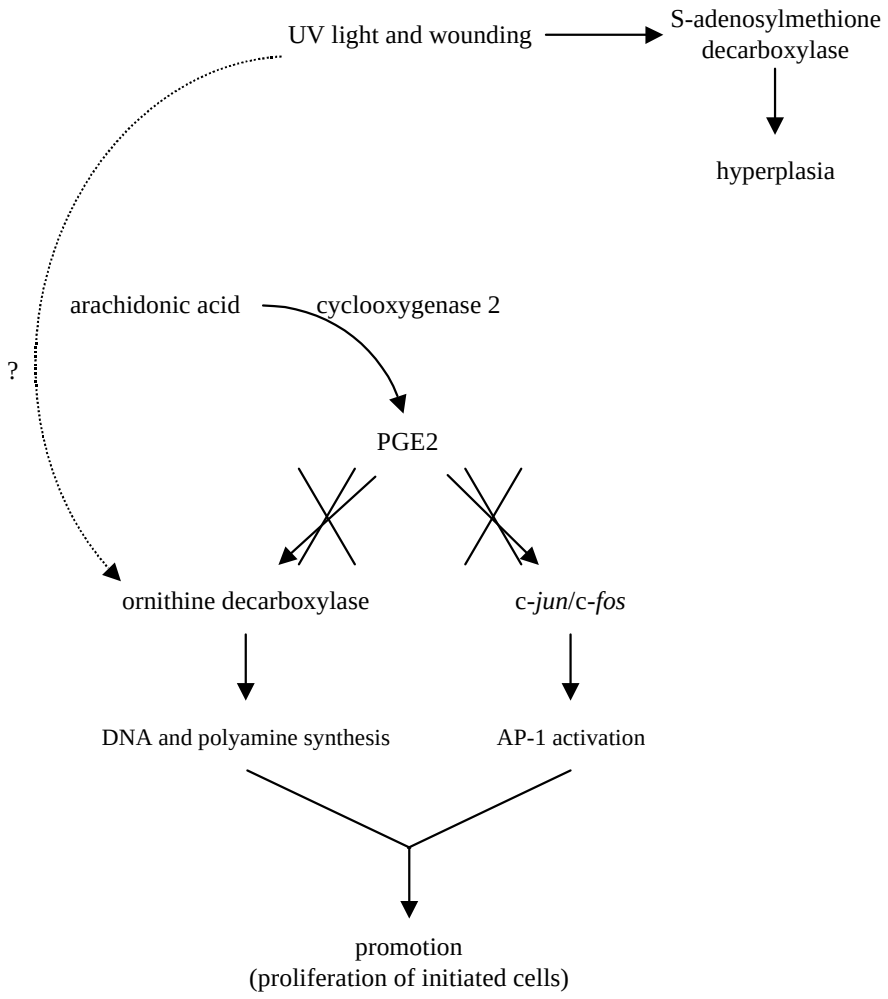


Figure 2-3
Proposed Mode of Promotion in Human Skin



3.0 Dose-Response Assessment

The epidemiological literature related to clinical use of pharmaceutical grade coal tar in the treatment of psoriasis or other skin conditions is most relevant to the risk assessment of the use of shampoos containing pharmaceutical grade coal tar. The clinical epidemiology studies used pharmaceutical grade coal tar ointments or gels; therefore, the correspondence between these varieties and the coal tar in shampoo is much better than the correspondence between coal tar in shampoo and the materials to which occupational cohorts were exposed. Furthermore, the dermal nature of the exposures in the clinical studies matches perfectly the exposure route of interest with coal tar-containing shampoos. Also, the concentration of coal tar used in shampoos and in ointments for psoriasis patients is in the same percentage range (1% to 3-5%) and the duration of usage is likely to be similar and linked to the duration of the skin condition. This is not the case with the occupational studies, where inhalation (and perhaps ingestion) of constituents or coal tar-containing materials would be common. The irritation effects seen with so many of the materials to which occupational cohorts were exposed are absent in the clinical studies and in the shampooing scenario of interest. Therefore, given this excellent correspondence between the clinical epidemiology studies and the shampoo-use exposures of interest, the natural choice is to develop risk estimates from the clinical epidemiology results.[2] This section documents that development.

The Pittelkow et al. (1981) study forms the basis for the risk assessment, with supporting information obtained from some of the other clinical studies. However, the consensus conclusion from all the clinical studies is that there is no clear evidence that the dermal application of pharmaceutical grade coal tar for treatment of psoriasis results in an increase in skin cancer incidence. However, for this quantitative assessment, it was presumed that lifetime exposure to

[2]As discussed in the previous section, the use of the available mouse skin painting studies for quantitative estimates of human skin cancer risk for coal tar-containing shampoos is not appropriate. Use of epidemiological data to assess human skin cancer risk is the only appropriate choice when evaluating coal tar-containing shampoos. However, for comparative purposes only, an analysis was conducted that attempted to incorporate the existing quantitative information on the pharmacokinetic differences between mouse and human skin. This risk assessment, as well as the results of an uncertainty analysis associated with the use of mouse skin data for human health risk assessment, are provided in Appendix E for comparison only.

pharmaceutical grade coal tar could result in an increased incidence of skin cancer. The use of the negative results from the Pittelkow study entails that the dose-response analysis to follow is very much a hypothetical "worst-case" analysis. Consequently, use of data from the Pittelkow study will result in potency estimates that are upper bound.

The issue of the "power" of the clinical studies to detect small increases in risk is not a relevant concern here. The conclusion of no risk is not based on a single study but rather is the consensus across a variety of studies. Moreover, in the same way that limited sample sizes (e.g., 260 individuals followed up in the Pittelkow et al. study) contribute to low "power," limited sample sizes also make the following dose-response analysis more conservative (health protective).[3]

3.1 Use of Pittelkow et al. (1981) Study for Dose-Response Modeling

Of the epidemiology studies reported in the literature for patients treated with coal tar, the study by Pittelkow et al. (1981) provided the most complete information with which to characterize coal tar exposure. The data from the Pittelkow et al. (1981) study provided sufficient information about the cohort, including information on the coal tar treatment regimens for the cohort (e.g., number of days/year, number of years of treatment), such that estimates of possible total coal tar exposure in this population (expressed as an uncertainty distribution) could be developed. Further, a distribution for the incidence of persons with skin cancers in this

[3]To understand how this is so, consider the following hypothetical example. Suppose that there are two studies that are comparable in all respects with the exception of sample size. Study 1 has a sample size of 100; Study 2 has a sample size of 50. The observed rates of response are 10/100 in Study 1 and 5/50 in Study 2, i.e., both have a 10% response rate. Suppose further that the expected number of responders in Study 1 was 5.3. Because all things except sample size are assumed equal, the expected number in Study 2 would be 2.65 (half the sample size, so half the expected number of responders) so that both studies report an expected response rate of 5.3% and a relative risk of about 1.89 (10%/5.3%). One test of the hypothesis that there is no risk (relative risk equal to 1) is to derive confidence bounds on the true response rate, based, of course, on the observed rate of 10%. Assuming that the observed numbers arise as a consequence of an underlying Poisson distribution (the typical assumption for incidence data), a one-sided, lower 95% confidence bound can be estimated for each study. In the case of Study 1, the lower bound is 5.45%; in the case of Study 2 it is 3.94%. Compared to the expected response rate of 5.3%, we would reject the hypothesis of no risk for Study 2 (the lower bound of 5.45% is greater than the expected value of 5.3%) at the 95% confidence level, but we could not reject that hypothesis based on the results of Study 2. Conversely, the calculation of one-sided 95% upper confidence bounds for response rate yields a value of 16.95% for Study 1 and 21.0% for Study 2, with corresponding relative risk estimates of 3.2 and 4.0, respectively. The use of a smaller study, although lessening the chances of rejecting the null hypothesis of no risk, yields the more protective upper bound on relative risk.

population could be determined, as could a distribution for the expected incidence of persons with skin cancer based on background rates. A Monte Carlo analysis was applied to the input distributions to yield an output distribution for coal tar potency, from which a conservation (health protective) high-end estimate was selected. The selected potency estimate was then used in a lifetable (age-adjusted) analysis to estimate the lifetime average coal tar exposure corresponding to the target extra lifetime risk level (one in 100,000).

3.1.1 Description of the Pittelkow et al. (1981) Study

In this study, a description of 260 psoriasis patients who had received at least one course of the Goeckerman regimen at the Mayo Clinic and who may have subsequently used coal tar preparations at home to control their psoriasis was provided. Pittelkow et al. (1981) conducted a retrospective study of medical records of all patients hospitalized for psoriasis, who underwent the Goeckerman treatment for the first time during 1950-1954, and reported the results of a 27-year follow-up of this initial population (mean duration of follow-up was 20.1 years). Information obtained from the Mayo Clinic included demographic data, skin pigmentation and hair color, medical history of malignant or premalignant conditions, and other medical treatments (arsenicals, methotrexate, or ionizing or UV radiation) that would tend to increase the carcinogenic risk in this population. The cumulative outpatient use of coal tar medications after initial Goeckerman therapy was recorded.

The incidence of skin cancer since the initial Goeckerman treatment in this population was recorded and, when possible, biopsy slides from the local physician were reviewed by a dermatologist to confirm the diagnosis. Of the 260 patients, 19 patients reported development of either a BCC or an SCC (total number of skin cancers reported was 32) (Table 3.1-1). The ratio of BCCs to SCCs was approximately 3:1. More than half of the BCCs were in sun-exposed areas of the body and 86% of the SCCs were on the head, neck and upper extremities. In all, 66% of the nonmelanomatous skin cancers were confined to sun-exposed areas of the body. The median age of patients with skin cancer was 56 with a range of 39 to 92 years. The distribution of age among patients in whom skin cancer developed subsequent to treatment was similar to the age distribution in the remaining 241 patients without skin cancer. For those who developed skin

cancer, the median number of years between initial treatment and the development of the skin cancer was 20 years, with a range of 4 to 27 years.

The characteristics of the patients with and without skin cancers were comparable (Tables 3.1-2 and 3.1-3). Neither group had a history of occupational exposure to known carcinogens. The tendency to sunburn with moderate sun exposure was the same for both groups. Premalignant skin conditions present prior to initiation of the Goeckerman treatment were reported in 10% of patients with skin cancer and 4% of those without skin cancer. More of the patients with skin cancer (67%) reported receiving repeated ionizing radiation treatments than those without skin cancer (58%).

Of the patients with skin cancer, 42% lived in sunbelt states, while 27% of those without skin cancer lived in sunbelt states. The median duration of years lived in the sunbelt was 14 and 15 years for patients with skin cancer and those free of skin cancer, respectively (Table 3.1-3).

In this cohort, 14% of the 260 patients who were included in the follow-up were admitted to the Mayo Clinic again in the period 1956-1960. This agrees with the reported revisitation rate for a larger patient group (approximately 3000 patients) treated at the Mayo Clinic over a 60-year period (Muller and Perry 1984), for which approximately 85% of the patients received only one treatment regimen, while 13% returned for a second treatment. Overall, the average number of treatments for the group reported in Muller and Perry (1984) was 1.5 hospitalizations. One individual was reported to have returned 16 times for the full Goeckerman treatment.

In the Pittelkow et al. (1981) cohort, coal tar usage during the Goeckerman therapy and during subsequent home use was comparable for those individuals who developed skin cancer and those who did not. The median years of at-home treatment were 21 and 14 for persons with skin tumors and those without skin tumors, respectively (Table 3.1-2). The percentages of persons using coal tar treatment at home were 56% and 72% in patients with and without skin cancer, respectively, and of those using home treatment, 67% and 56%, respectively, used the product at home for 50 days or more per year (Table 3.1-3).

According to the authors, the mean duration of follow-up was 20.1 years and ranged from 2 to 28 years and the total person-years (the sum of the follow-up times for all patients) was 5,222. Pittelkow et al. (1981) used age-specific incidence rates from the TNCS (Scotto et al.

1974) to calculate the number of persons in whom skin cancer would be expected to develop. An expected number of 26.6 persons was calculated as the average of expected numbers from four geographic areas (49.2 for Dallas-Fort Worth; 15.5 for Iowa; 18.7 for Minneapolis-St. Paul; and 23.1 for San Francisco).

3.1.2 Estimate of Exposure to the Subjects Evaluated in the Pittelkow et al. (1981) Study

The data provided in the Pittelkow et al. (1981) study along with other reports of coal tar usage at the Mayo Clinic (Muller and Perry 1984) and other clinics (Menter and Cram 1983) were used to develop estimates of exposure to this population. The patients in the study populations reported by Muller and Perry (1984) and Menter and Cram (1983) were also exposed to coal tar according to the Goeckerman regimen employed for the Pittelkow et al. (1981) patients. A description of this therapy regimen was discussed in Section 2.2.2.

The goal of this exposure estimation was to estimate an average exposure for the entire cohort of 260 individuals. The manner in which data were reported in Pittelkow et al. (1981) does not allow that average exposure to be estimated without uncertainty. Consequently, the average exposure was treated as an uncertain parameter represented, not as a point estimate, but as a distribution of possible values. The distribution of possible average exposure estimates was calculated, as described below, based on uncertain determinants (input parameters) of the exposure scenario, each of which was described by its own distribution of possible values. A Monte Carlo analysis allowed the combination of the uncertain input parameters, yielding the distribution of the average exposure estimate. Details of the calculations are provided here.

For any individual in the cohort, his or her total exposure is given by

$$E_{tot} = E_1 + E_2 + E_h$$

where,

E_{tot} = Total Exposure (grams)
E_1 = Exposure from hospital treatments during the period from 1951-1955 (grams)
E_2 = Exposure from hospital treatments during the period from 1956-1960 (grams)
E_h = Exposure from at-home treatments (grams)

The average exposure for the cohort as a whole is the sum of the E_{tot} values for all the individuals, divided by 260. Therefore, to derive the desired distribution of possible values of average exposure, we needed to determine distributions of possible values of E_1, E_2, and E_h for each of the individuals in the cohort.

A subset of the study population was cross-classified by Pittelkow et al. (1981) in terms of two factors, the first being whether or not the patient developed skin cancer, and the second being the "treatment characteristics," summarized into three categories: 1) coal tar therapy only at the Mayo Clinic (no home use); 2) coal tar therapy at the Mayo Clinic and follow-up at-home usage at a rate of less than 50 days/year; and 3) coal tar therapy at the Mayo Clinic and follow-up at-home usage at a rate of 50 days or more per year. For each of the six resulting cross-classifications, Pittelkow et al. (1981) provided data on the number of individuals (derived from data in Table 3.1-3), as well as data on the median number of days of coal tar treatment both in the hospital and at home, and the median number of years of treatment (Tables 3.1-2 and 3.1-3). These data were used to derive estimates of exposure.

The subset of the study population for which the data described above was available included 16 of the 19 patients with skin cancer and 125 of the 241 patients without skin cancer. For the 16 skin cancer patients, seven received coal tar therapy only at the clinic, three had at-home treatment at a rate of less than 50 days/year, and six had at-home treatment at a rate of 50 days/year. Similarly, for the 125 patients without skin cancer, 35 received coal tar therapy only at the clinic, 40 had at-home treatment at a rate of less than 50 days/year, and 50 had at-home treatment at a rate of 50 days or more per year. For the 42 (=7+35) patients with no at-home treatment, it was known that $E_h = 0$. For the remaining patients, the distribution of nonzero values for E_h was derived as described below.

There were three patients who developed skin cancer and 116 patients who did not develop skin cancer for whom the treatment characteristics were unknown. To complete the Monte Carlo analysis (requiring an exposure estimate for each member of the cohort) these patients had to be classified according to treatment characteristics. To reflect the uncertainty concerning the treatment characteristics for these 119 patients, each iteration of the Monte Carlo procedure randomly classified these patients with respect to treatment characteristics. For the

three with skin cancer, the probabilities that they had clinic only, less than 50 days/year at-home, or more than 50 days/years at-home treatment were 7/16, 3/16, and 6/16, respectively, i.e., the rates at which the skin cancer patients with known treatment characteristics fell into the three categories. Similarly, for the 116 without skin cancer, the probabilities that they had clinic only, less than 50 days/year at-home treatment, or more than 50 days/year at-home treatment were 35/125, 40/125, and 50/125, respectively, i.e., the rates at which the patients without skin cancer with known treatment characteristics fell into the three categories.

Additionally, it was known that 36 patients, six who developed skin cancer and 30 who did not, had hospitalizations for Goeckerman treatment at the Mayo Clinic during the period from 1956 to 1960. It was unknown which patients those were. To reflect this uncertainty, each iteration of the Monte Carlo procedure randomly assigned a nonzero value for E_2 (see the description of this variable below) to six of the patients with skin cancer and to 30 of the other patients. For all other patients, E_2 was set equal to zero.

It is important to note that in the following derivations of exposure to coal tar, prior exposures to coal tar and use of coal tar-containing shampoos have not been included in the calculations. Pittelkow et al. (1981) reported that more than 67% of their patients had had prior coal tar exposures (see Table 3.1-3). Moreover, Muller and Perry (1984), describing the Goeckerman treatment at the Mayo Clinic**,** discuss the use of coal tar-containing shampoos as part of the in-hospital therapy. It is very likely that the members of the Pittelkow et al. (1981) cohort used such shampoos either in the hospital setting or at home in conjunction with the at-home treatment of their psoriasis. Ignoring these other sources of coal tar exposure entails that the calculations presented below have underestimated the total coal tar exposure for the Pittelkow et al. (1981) cohort and therefore overestimated the potency of coal tar with respect to skin cancer. The overestimation of the potency in turn entails an underestimate of the NSRL, i.e., a NSRL estimate that is lower than it otherwise should be.

3.1.2.1 Estimates of Exposure During Treatment at the Hospital

Estimates of the amount of coal tar exposure that could have occurred during the initial and any subsequent hospital stay were estimated using the following equation:

$$E_{1,2} = \%CT \times G \times D_{t1,t2} \times F$$

where,

$E_{1,2}$	=	Exposure from hospitalizations during either 1951-1955 (1) or 1956-1960 (2) (in grams)
%CT	=	% coal tar in ointment (unitless)
G	=	Grams of coal tar in a 1% ointment required to cover the treated surface area during a single day's treatment (g/day)
$D_{t1,t2}$	=	Days of treatment in hospital for period 1 or 2
F	=	Fraction of applied coal tar absorbed

Percentage of Coal Tar Used in Hospital Therapy (% CT)

Various coal tar preparations, with coal tar concentrations ranging from 2% to 10%, have been used in psoriasis therapy (Dodd 1993; Maughan et al. 1980; Muller and Perry 1984; Perry et al. 1968). Muller and Perry (1984) stated that a 2% coal tar ointment was used at the Mayo Clinic during the years when the members of the Pittelkow et al. (1981) cohort were treated. Maughan et al. (1980) reported a companion study to the Pittelkow et al. (1981) cohort for patients also treated at the Mayo Clinic during 1950-1954. The concentration of coal tar in the ointment for this population was 2%. It was assumed that the patients in the Pittelkow et al. (1981) study were treated with 2% coal tar ointment. This value was used as a point estimate for this parameter in the exposure assessment.

Estimates of Grams of Coal Tar Used During A Hospital Visit (G)

Muller and Perry (1984) stated that the average amount of 2% coal tar applied during the Goeckerman therapy at the Mayo Clinic was estimated to result in about 3 grams of coal tar/day (approximately 150 grams of ointment applied/day), but the range of the amount of coal tar applied was not given. However, as described in Section 2.2.2, the treatment regimen called for total body coverage (with the exception of the face, genitals, and axillae). Consequently, the total amount applied would vary with each patient and would be a function of the height and weight of that patient, for which body surface area was considered a reasonable surrogate. Therefore,

estimates of the grams of coal tar exposure per day for each patient were calculated by scaling to the body surface area.

First, the average value of 3 grams of coal tar (for a 2% ointment) was normalized to a 1% coal tar ointment; i.e., an average of 1.5 grams of coal tar per percentage of coal tar in the ointment applied. Next, data on the body surface areas (excluding head, genitals, and axillae) of men and women were obtained from the *Exposure Factors Sourcebook* (AIHC 1994);[4] the mean value was μ=1.84 m^2 (with σ= 0.23, min=1.4, and max=2.3). It was assumed that a person with an average body surface area (1.84 m^2) would have received the average normalized application of 1.5 grams of coal tar, leading to an estimate of 0.815 grams of coal tar applied per m^2 per percent of coal tar in the ointment. It was assumed that this ratio applied for all body surface areas. A distribution for the normalized daily exposure to coal tar when treatment occurred in the clinic was therefore obtained by multiplying 0.815 times the normal distribution of body surface areas. That is, each iteration of the Monte Carlo simulation sampled a body surface area for any given patient (from the normal distribution with mean 1.84 and standard deviation 0.23) and multiplied that sampled value by 0.815. The resulting distribution was the distribution for grams of coal tar used per day in the hospital phase of treatment, normalized to a 1% coal tar ointment (Table 3.1-4).

Days of Treatment During the Hospital Treatment ($D_{t1,t2}$)

Pittelkow et al. (1981) reported that the median number of days for hospital treatments in the periods 1951-1955 and 1956-1960 were 16 and 21 days, respectively, for the patients who developed skin cancer and 17 and 21 days, respectively, for the patients who did not. Muller and Perry (1984) reported that the average duration of a single hospital stay was 2-3 weeks for moderate cases and 3-4 weeks for severe cases. Menter and Cram (1983) stated that the range of days of Goeckerman treatment at two psoriasis centers was 8-38 days.

For the Monte Carlo analysis, the distributions for days of treatment in the hospital were defined as follows. For days of exposure during the first period of hospital visits (1951-1955),

[4]Use of these data rather than the *Exposure Factors Handbook* (USEPA 1997) provided a wider distribution of surface area values and, consequently, a lower lower-bound on the amount applied and was based on a larger number of individuals.

the range of discrete values was assumed to run between 8 and 38 days. At the minimum of 8 days, the probability of such an exposure duration was 0.022, rising to 0.049 for 13 days, and falling again to 0.002 for 38 days. This distribution was selected so that the durations closer to the center of the range were more likely than durations at the extremes of the range and so that the calculated median value matched that reported by Pittelkow et al.(1981), i.e., roughly 17 days (Table 3.1-5). Similarly, for the second period of clinic visits (1956-1960), the custom distribution had a range of discrete values between 8 and 38 days, the probability of an 8-day exposure was 0.005, the peak probability was 0.044 at 15 days, and the probability of a 38-day exposure was 0.007. Again, the particular choices yielded a match between the calculated median and the median reported by Pittelkow et al. (1981) of 21 days (Table 3.1-5). Note that only 36 members of the cohort had hospital visits during the period 1956-1960. Appendix B provides details of these distributions.

For both of the distributions defined above, the lower bound of 8 days is supported by the literature (Muller and Perry 1984; Menter and Cram 1983). Indeed, Muller and Perry (1984) describe the procedure employed at the Mayo Clinic (where members of the Pittelkow et al. cohort were seen) and state that certain UV treatment options are not implemented until after 8-10 days of therapy. The upper bound of 38 days is based on the experience in psoriasis day care centers (Menter and Cram 1983) and is for a single set of treatments. Because it is entirely possible that the members of the Pittelkow et al. (1981) cohort had more than one hospitalization during either of the time periods, the value of 38 may be too low as an upper bound on the number of days of hospital treatment. However, in the absence of further information, the upper bound of 38 has been used and it is noted that had alternative upper bounds greater than 38 been used, the effect would be to increase the calculated average exposures for this cohort, leading to a decrease in the estimated potency, and an increase in the NSRL.

Fraction of Applied Coal Tar Absorbed (F)

The fraction of applied coal tar that is absorbed from the ointments applied to the members of the Pittelkow et al. (1981) cohort is itself an uncertain value (one that varies from individual to individual). The parameter *F* is defined in terms of three other parameters that have

been estimated from the literature. Because the values of those parameters are uncertain, and are represented by uncertainty distributions as described here, the parameter *F* has a composite distribution determined by the distributions of the three parameters in question.

For this analysis, *F* has been defined as follows:

$$F = \frac{P_e / P_a}{C_f}$$

where

P_e = amount of pyrene excreted as the 1-OH-P metabolite (µmol)
P_a = amount of pyrene applied in an ointment (µmol)
C_f = a conversion factor specifying the fraction of absorbed pyrene that is metabolized to and excreted as 1-OH-P (unitless).

In the above, pyrene, a PAH constituent of coal tar, is used as a marker of absorption of coal tar as a whole. That is, the fraction of applied pyrene that is absorbed is taken as a surrogate (estimate) of the fraction of applied coal tar constituents that are absorbed. The goal of this analysis of *F* is the derivation of distributions for P_e, P_a, and C_f that can be considered generalizations to psoriasis patients treated in the manner of Goeckerman.

A study by Santella et al. (1994) is exactly the type of experiment needed to provide that generalization for the parameters P_e and P_a. That study examined 57 psoriasis patients undergoing a Goeckerman-type treatment, as did the members of the Pittelkow et al. (1981) cohort being analyzed. Those patients had at least 2 days of in-patient Goeckerman treatment. The excretion of 1-OH-P was monitored for a full 24 hours; Santella et al. (1994) reported that the mean and standard deviation of the excretion values were 546 and 928 µmol/mol creatinine, respectively, converted to 6.77 and 11.51 µmoles, respectively, by assuming that the daily amount of creatinine excreted was 0.0124 mol. Santella et al. (1994) reported that a log-transformation of the data made them more symmetric, suggesting that a lognormal distribution would be the appropriate characterization of the variability in 1-OH-P excretion following the defined exposures. It was assumed that a lognormal distribution with a mean and standard deviation of 6.77 and 11.51 µmoles, respectively, could be used to describe the uncertainty for P_e in a population of psoriatics applying coal tar-containing ointment.

The same type of generalization of the parameter P_a was also required, and was obtained by making the following assumptions. Because Santella et al. (1994) reported only a range for the amount of pyrene applied (20-100 grams of coal tar/day, which corresponds to 69-346 µmoles pyrene/day) that was self-administered, that range was compared to the range of 1-OH-P excretion (0.12-64 µmoles). Based on that comparison, the distribution for P_a was "scale-up" from the distribution assumed for the 1-OH-P excretion. That is, the distribution for P_a was also assumed to be lognormal. The standard deviation for that lognormal distribution was scaled according to the ratio of the ranges, since the range is an indicator of and varies as a function of the variability. Finally, the mean value was assumed to fall at the same relative location within the range as did the mean for 1-OH-P excretions (i.e., both were assumed to fall about 10.4% of the way between the minimum and the maximum). This scaled-up distribution was used as the uncertainty distribution for P_a, describing the generalized pattern of pyrene exposure among psoriatics being treated as described by Santella et al. (1994).

Several studies provide data relevant to the estimation of C_f. The available human data on the fraction of absorbed pyrene converted to and excreted as 1-OH-P vary greatly. Viau et al. (1995) estimated the fraction of orally administered pyrene that was excreted in the urine as 1-OH-P over 48 hours. The value of C_f estimated from Viau et al. (1995), assuming 100% absorption from ingested olive oil, is 0.0367. Based on absorption of pyrene in workers exposed to coke oven particulates, Van Rooij et al. (1993a) estimated the percent conversion to range between 13% and 49%.

Data that may be more representative of the exposures of interest are those from Jongeneelen and Bos (1990), Singh et al. (1995) and Van Rooij et al. (1993b). From these data it can be estimated that the conversion of absorbed pyrene to 1-OH-P in humans is virtually complete (90% assumed) and that most of the 1-OH-P formed is excreted in the urine (90%); if that is the case, then the conversion percentage would be estimated to be 81% (see Appendix C). Using the same estimate for urinary excretion (90%), but a lower estimate, based on rodent data (Jacob et al. 1989), for metabolic conversion (46%), Van Rooij et al. (1993b) estimated a conversion percentage of 41.4%.

The values of 3% and 81% may represent two ends of the spectrum of possible values for C_f. In fact, the distribution assumed for C_f in the Monte Carlo analysis was a uniform distribution from 0.03 to 0.81.

When all three of these distributions were sampled, a distribution for the absorption fraction *F* was obtained. It is obtained from the generalizations of the data from Santella et al. (1994) and the composite data relevant to the estimation of C_f, such that that distribution applies to a hypothetical population of psoriasis patients being treated in the manner described by Santella et al. (1994).[5] Making the reasonable assumption that the distribution for *F* that is derived in this manner can be applied to other cases of psoriatics applying coal tar-containing ointments to their skin, then the derived distribution for *F* is relevant to the members of the Pittelkow et al. (1981) cohort as well. The same distribution for *F* was used for the in-hospital phase (as described here) and for the ointment treatment at home (see next section). In fact, as long as it is assumed that C_f does not depend on the specifics of the dermal exposure scenario (which is also a reasonable assumption since the fate of pyrene in terms of its metabolism and excretion, just what is being determined by C_f, should be the same no matter how pyrene and coal tar are absorbed through the skin), the distribution for C_f can be used for individuals exposed to coal tar through shampooing (see Section 4.4).

3.1.2.2 Estimates of Exposure During At-Home Coal Tar Treatment

At-home usage was defined by the following equation:

$$E_h = \%CT_h \times G \times B \times D_h \times Y_h \times F$$

where,

E_h	=	Exposure from at-home treatment (grams)
$\%CT_h$	=	% coal tar in ointment used at home (unitless)
G	=	Grams of coal tar in a 1% ointment required to cover the whole body treatment surface area for one day's treatment (grams/day)

[5]The distributions were sampled independently from one another. In a small percentage of the sampling, the values selected would have led to an absorption fraction estimate greater than 1. Since this is a biological impossibility, such values were replaced with the value 1, i.e., it was assumed that for such individuals, 100% absorption would take place.

B	=	Fraction of body treated at home (unitless)
D_h	=	Days of at-home coal tar treatment/year (days/year)
Y_h	=	Years of at-home coal tar treatment (years)
F	=	Fraction of the applied coal tar that is absorbed

Percentage of Coal Tar Used for At-Home Treatment (% CT_h)

Although the percentage of the coal tar in the ointment used during hospital treatment for the Pittelkow et al. (1981) cohort treated at the Mayo Clinic could reasonably be assumed to be 2%, the percentage of the coal tar in the ointment used during at-home treatment could not be assumed to be a fixed value, based on information provided. At-home treatments are known to vary. A range of 1% to 25% is given for coal tar in ointments used on an outpatient basis (Dodd 1993; Kanzler and Gorsulowsky 1993; Leong 1990; Silverman et al. 1995). Both the frequency of use and the strength of the product used would be a function of the severity of the disease. While the Pittelkow et al. (1981) cohort was characterized as severely affected, with 85% of the cohort having severe or generalized psoriasis that required almost total body coverage with coal tar, patients could have opted to use coal tar ointment that was either a greater or lesser strength than that used in the hospital. Based on this information, and the fact that Kanzler and Gorsulowsky (1993) stated that relatively few of their patients were willing to use an ointment at home with more than 5% coal tar, the assumed distribution for percentage of coal tar in the ointment used at home was a uniform distribution with a range from 1% to 5%. This covers the range between the lowest concentration assumed to have therapeutic benefit and a concentration likely to be tolerated by patients in an at-home treatment (Table 3.1-6). The mean, median, minimum, and maximum values of the percent coal tar used at home derived by sampling from this distribution are given in Table 3.1-7.

Grams of Coal Tar in Ointment Used for At-Home Treatment (G)

For the in-clinic application of ointment, it was assumed that the grams of coal tar applied in one day (for a 1% coal tar ointment) was 0.815 times the body surface area (see Section 3.1.2.1 above). Muller and Perry (1984) state that at the Mayo Clinic (where members of the Pittelkow cohort were treated) the head, neck, and intertriginous areas were not treated. It is assumed that those areas account for 10% of the body surface area. Therefore, the average in-

clinic treatment (average amount applied to the person of average body surface area) consisted of 1.5 g coal tar over 90% of the body surface area (assuming a 1% coal tar ointment) or $1.5/(0.9*1.84) = 0.906$ g/m^2.

For at-home use, it was assumed that the same rate of application (g/m^2) applies. It is likely that this is an underestimate of the amount applied, because patients self-administering the ointment would tend to apply more than in the controlled clinic setting, in accordance with the dictum that "if a little is good then more is better." Nevertheless, under the assumption of the same application rate at home as in the clinic, the amount of coal tar that would be used to cover the entire body (assuming a 1% coal tar ointment) is obtained by sampling a body surface area (in m^2) from the normal distribution discussed above, and multiplying that surface area by 0.906. The fact that less than the entire body is treated at home is accounted for by the parameter *B*.

Fraction of the Body Surface Area Treated (B)

Patients released from the hospitalized portion of the Goeckerman therapy were in remission for variable lengths of time, after which at-home treatment would resume. Additional information on the percentage of patients who continue therapy either at home or on a day-care basis can be inferred from data on the length of remission following initial treatment (Durham and Morgan 1974). In 231 cases followed, less than 30% had complete remission and then only for an average duration of 3 months before resuming treatment (Lane and Crawford 1937). In another study, 74% of 400 patients had never been clear of the condition since the onset of the disease (Church 1958). Following coal tar treatment (260 patients), the majority of these patients were in remission for only 6 months before resuming treatment. Pittelkow et al. (1981) reported that 72% of patients without skin cancer continued at-home therapy for a median of 14 years post-hospital stay, while 56% of the patients with skin cancer continued at-home therapy.

While a considerable number of patients continued coal tar therapy at-home, the amount of coal tar assumed to be used was less, on the average, than would have been used during the hospital phase of treatment. For the at-home treatments, only a fraction of the body surface area was assumed to be treated. Pittelkow et al. (1981) stated that more than 85% of the 260 patients had severe or generalized psoriasis that required almost total body coverage during hospital

treatment. However, it was assumed that at-home treatment would be for something less than total body coverage. In Drake et al. (1993), the criteria for hospital admission included psoriasis covering more than 25% of the body surface. Consequently, it is likely that if more than 25% of the body was actively psoriatic, then the patient would seek a formal hospital treatment. Moreover, the tolerability of the ointment use suggests that a small percentage of the body would be treated, on average. Dermatologists familiar with the treatment of psoriasis with coal tar ointments report that home treatment of 25% of the body surface area is a reasonable upper bound (TERA review committee, 2000). Therefore, it was assumed that the maximum body surface area that would be treated at-home was 25%. A lower bound of 5% body surface area was assumed. A uniform distribution with a minimum of 5% and a maximum of 25% was used to represent uncertainty concerning the fraction of the body surface that was treated during at-home treatments (Table 3.1-6). The mean body surface area treated associated with these assumptions was 15% of the body (Table 3.1-7).

Days of At-Home Treatment (D_h)

At-home treatments would be expected to vary depending on the level of involvement and severity of the psoriasis. Maughan et al. (1980) reported that for patients treated at the Mayo Clinic, at-home treatment ranged from zero days to every day for 26 years.

For the Pittelkow et al. (1981) cohort, days of at-home use per year were estimated separately for the group who reported a rate of use of less than 50 days/year and for the group who reported a rate of use of 50 days or more/year. As described above, those individuals for whom this reporting was unavailable were assigned to one of these groups (or to the no at-home treatment group) randomly for each iteration of the Monte Carlo simulation, and so they were treated appropriately depending on that assignment. For those with an at-home treatment rate of less than 50 days treatment/year, it was assumed that the number of days of exposure would range from 10 up to 49 days/year. For the other group, at-home treatment could range from 50 to 365 days/year. Therefore, two discrete uniform distributions (taking only integer values) were used (Table 3.1-6). For patients who treated at home for less than 50 days/year, a uniform distribution with the lower and upper limits of 10 and 49 days was used. For the remaining

patients who treated with coal tar at home, a uniform distribution with lower and upper limits of 50 and 365 days was used. For persons in the less than 50 days/year treatment category, the mean number of days/year estimated using Monte Carlo analysis was 30 (Table 3.1-7) or slightly more than one day every other week. For those persons with at least 50 days of at-home treatment/year, the mean number of days of treatment was 208 or approximately 4 days/week (Table 3.1-7).

Years of At-Home Treatment (Y_h)

Pittelkow et al. (1981) reported that the median value of at-home use was 21 years for the patients who developed skin cancer and 14 years for the patients who did not develop skin cancer. Pittelkow et al. (1981) also stated that the median number of years from the initial Goeckerman treatment to the development of a skin cancer was 20 years with a range of 4 to 27 years. It is recognized that cumulative exposure throughout follow-up would not be the appropriate dose metric for an individual who developed skin cancer but continued coal tar treatment. It is recognized that continued exposure after the development of the skin tumor would not contribute to the development of that tumor. Therefore, because the median years to tumor was similar to but slightly less than the median years of at-home treatment (for those individuals who developed skin cancer), the distribution for years of home use among those patients was equated with the distribution of years to development of a nonmelatomatous skin cancer. The discrete triangular distribution (taking only integer values) with a minimum, a most likely value, and a maximum of 4, 26, and 27 years, respectively, was used as the distribution of years of home treatment for the patients who developed skin cancer.

For the patients who did not develop skin cancer, the assumed discrete triangular distribution for years of at-home use had a minimum, a most likely value, and a maximum of 1, 14, and 27 years, respectively (Table 3.1-6). The values obtained by sampling from these distributions (used in the Monte Carlo analysis) are summarized in Table 3.1-7. The mean estimated years of at-home treatment were approximately 19 and 14 years for patients who developed skin cancer and those who did not, respectively.

3.1.2.3 Estimates of Total Exposure

The Monte Carlo analysis consisted of multiple (300,000) iterations of sampling from the distributions described above, and calculation of each individual's total exposure, E_{tot}. Thus, for each iteration an average over the 260 individuals was determined, and the distribution of the average values was used in the dose-response assessment as described in the following section to derive bounds on the doses associated with specified levels of risk. The average total exposure distribution had a mean of 254 g absorbed and a median of 252 g absorbed.

3.1.3 Dose-Response Analysis Using Pittelkow et al. (1981)

The data from Pittelkow et al. (1981) were used to derive estimates of "potency factors" that can be used in a lifetable analysis of nonmelanoma skin cancer risks. The basic relationship assumed here is the following:

$$\mathrm{RR} = 1 + \mathrm{b} \times \mathrm{d},$$

where *RR* is relative risk, *b* is the potency factor to be estimated, and *d* is cumulative exposure. This equation specifies that the relative risk (the probability of getting nonmelanoma skin cancer for an individual having cumulative exposure *d*, divided by the probability of getting nonmelanoma skin cancer for an individual having no exposure) is a linear function of cumulative exposure. The linear model was chosen for two reasons. First, a linear model for cancer dose-response has for many years been the default choice of USEPA cancer risk assessment. Second, with a single exposed group available for analysis, only a single parameter can be estimated. The linear model is the natural single-parameter model to choose in the absence of other information.

Rearranging the equation above yields

$$b = (RR - 1) / d$$

showing how *b* can be estimated from information about the values of *RR* and *d*. The previous discussion (Section 3.1.2) described how distributions of average total (or cumulative) exposure

for the Pittelkow et al. (1981) cohort have been determined. Therefore, in order to complete the analysis and derive potency factor estimates, values for *RR* are required.

Pittelkow et al. (1981) used data from the TNCS (Scotto et al. 1974) to calculate the expected number of persons with a nonmelanoma skin cancer in the study population of 260 patients. Pittelkow et al. (1981) calculated the expected number of persons with skin cancer to be 49.2 based on Dallas rates, 23.1 based on San Francisco-Oakland rates, 18.7 people on Minneapolis rates, and 15.5 based on Iowa rates. The average of these four expected numbers is 26.6. Using this average value, the probability of getting nonmelanoma skin cancer for an individual having no exposure [but who is otherwise similar to an individual in the Pittelkow et al. (1981) cohort, for example, with respect to age and gender] was estimated as 26.6/260.

However, because the places and durations of residence of the members of this cohort were not known, there is uncertainty concerning the appropriate value of the expected numbers contributed by each cohort member. To represent that uncertainty, a normal distribution was selected. The normal distribution had a mean of 26.6, a standard deviation of 3.5, and was truncated at 15.5 and 49.2, the extremes of the values reported by Pittelkow et al. (1981). For the Monte Carlo analysis, it was assumed that each individual contributed towards the expected number equally: for each iteration of the simulation, an individual's contribution towards the expected number was determined by sampling from the normal distribution just defined and dividing by 260. Summing over all individuals yielded an expected number for the cohort as a whole of between 15.5 and 49.2, i.e., a probability of getting nonmelanoma skin cancer of between 15.5/260 and 49.2/260. A total of 300,000 estimates for the total expected number was obtained in the Monte Carlo analysis.

Note that the use of a normal distribution in the above calculations that has a mean of 26.6 gives equal weight to all four of the reported expected numbers. That is, the mean value is determined as if all of the reported expected numbers were equally relevant to the residence pattern of the Pittelkow et al. (1981) cohort. In fact, however, some rough approximations suggest that the rates from sunbelt locations (i.e., Dallas-Fort Worth with an expected number of 49.2) might deserve greater weight. Consider that Pittelkow et al. (1981) report that eight individuals who developed skin cancer resided in the sunbelt with a median residence time of 14

years and that 58 individuals who did not develop skin cancer lived in the sunbelt with a median residence time of 15 years (Table 3 of Pittelkow et al.). Since this table reports medians for those individuals "possessing the given characteristic" it can be inferred that the remaining individuals did not reside in the sunbelt. Assuming that the information on location and years of residence could only have been obtained from those individuals who responded to questionnaires or phone interviews (estimated to be a total of 141 based on responses to questions about use of tar at home – Table 2 from Pittelkow et al.), the mean number of follow-up years spent in the sunbelt can be estimated to be 7.0 per individual (8*14 + 58*15/141 = 7.0 years, on average, in the sunbelt). If we assume that this rough calculation is indicative of the pattern for the entire cohort and considering that the average duration of follow-up was 20.1 years, then one would estimate that about 35% (7/20.1) of the years of follow-up for the cohort were lived in sunbelt locations. The implication of these rough calculations is that the normal distribution used to represent uncertainty about the expected number of skin cancers may underestimate that expected number.[6] The effect of underestimating expected numbers is to overestimate the potency of coal tar with respect to nonmelatomatous skin cancer.

The best estimate of the probability of response in the Pittelkow et al. (1981) cohort is 19/260. But that estimate is subject to uncertainty, an uncertainty that might ordinarily be reflected in terms of confidence limits. In fact, making the standard statistical assumption that the observed number of cases arises (with sampling error) from an underlying Poisson random variable, the 95% upper bound on the probability of response with 19 observed cases is 27.9/260. This upper limit is obtained by treating $k=19$ as if it comes from a Poisson distribution with expected value $t = 19$. The upper limit on the quantity $2rt$ is where $r = k/t$ is obtained by taking

[6]An alternative for the normal distribution that gives greater weight to the expected numbers from sunbelt states was not implemented in this analysis, at least partly for the following reason. The exact timing of the years of residency in the sunbelt is unknown. If some portion of the residence in that region occurred after the occurrence of a skin cancer, for example, then the determination of expected numbers of people with skin cancer would not include those years of residence in the calculations. Similarly, if residence in a more northern location, with lower expected rates of skin cancer, occurred after occurrence of skin cancer, say to avoid sun exposures known to be associated with development of such cancers, then the lower rates of such residence times should not be included in the expected number calculations. Obviously, the timing-of-residence issue is complicated, and not amenable to resolution with the information now available to us, so the most straightforward treatment, assuming equal contributions from all locations, has to be the preferred approach. Nevertheless, the uncertainty about such an assumption is addressed further in Section 5.0, Risk Characterization.

2rt to have a chi-square distribution with *2k+2* degrees of freedom (Cox and Oakes 1984). Note that *2rt = 2t*. This results in the equation:

$$t_u = \frac{1}{2} \times \chi^2(0.05, 2k+2) = 27.9,$$

where t_u is the 95% upper bound on the expected value and ÷²*(á,u)* is the *(1-á)th* percentile of the chi-square distribution with *u* degrees of freedom (Cox and Oakes 1984). In this case, ÷²*(á,u)* is ÷²*(0.05,40)* = 55.78.

For this analysis, the distribution of possible estimates of the true probability of response was determined. That is, the likelihoods that specific values were actually the true probability of response were estimated. For example, to one decimal place accuracy, the likelihood that the true probability of response is 19.0/260 (the most likely value) is 0.009112, that the true probability of response is 19.1/260 is 0.009110, etc. In the Monte Carlo simulation, a specific value was sampled for each iteration, where the probability of sampling a particular value was equal to the likelihood that that value was the true value, having observed 19 responses out of 260. Operationally, the selection was cut off at 39.9/260; the likelihood that the true value is greater than 39.9 is less than 2 in 10,000. The sampling from the distribution for probability of response was done 300,000 times.

To complete the analysis, each of the 300,000 estimates of the probability of response was divided by randomly selected values from the 300,000 estimates of the probability of response if no exposure had occurred to yield 300,000 values of *RR*. These were randomly paired with 300,000 estimates of the average cumulative dose (derived as described above, Section 3.1.2 from a distribution of E_{tot}) to get 300,000 estimates of the potency parameter *b*.

The 95th percentile of those potency parameter estimates can be considered a 95% confidence limit on *b*, labeled here b_u, reflecting uncertainty in both of the input parameters, *d* and *RR*. The value of b_u determined by these methods was $b_u = 1.8 \times 10^{-4}$/gram absorbed.

To use this specific, conservative estimate derived from Pittelkow et al. (1981) data, a lifetable approach is used to estimate the average daily dose that would give a lifetime risk equal to one in 100,000. It is assumed that the relative risk function

$$RR = 1 + b \times d$$

with $b = b_u = 1.8\times10^{-4}$/gram is appropriate for all ages. In that case, the lifetime probability of developing a nonmelanoma skin cancer, *LTR(D)*, is approximated by

$$LTR(D) = \sum_{i=1}^{15} (Pr_i \times c_i \times \Delta_i) \times f_i(D)$$

with

$$LTR(0) = \sum_{i=1}^{15} P_i \times c_i \times \Delta_i$$

where P_i is the probability of surviving without nonmelanoma skin cancer until the beginning of the ith age interval for the unexposed population, Pr_i is the probability of surviving without nonmelanoma skin cancer until the beginning of the ith age interval for the exposed population , c_i is the incidence rate for nonmelanoma skin cancer in the ith age interval, and $f_i(D)$ is the relative risk of getting nonmelanoma skin cancer during the ith age interval from exposure to *D* grams/year of coal tar. Each of these parameters is conditional on surviving without nonmelanoma skin cancer until the beginning of the interval and $\ddot{A}_i$ is the width of the ith age interval. In this approach, age was divided into 15 age categories, <1, 1-4, 5-9, 10-14, 15-19, ..., 65-69. Because the exposure assessment of interest (coal tar-containing shampoo use) specifically mandates an assumed 70-year lifespan, the lifetable analysis ends at age 70.

P_i and Pr_i were calculated recursively using the 1989 death rates from all causes [USDHHS (1989), using rates for Caucasians, both genders combined]. $P_1 = Pr_1 = 1$. The recursion formula for P_i is

$$P_i = P_{i-1} \times (1 - \Delta_{i-1} \times (r_{i-1} + c_{i-1})) \text{ for i = 2,…,20}$$

and the recursion formula for Pr_i is

$$Pr_i = Pr_{i-1} \times (1 - \Delta_{i-1} \times (r_{i-1} + c_{i-1} \times f_{i-1}(D))) \text{ for i = 2,…,20}$$

where r_i is the yearly death rate for all causes for the ith age interval, c_i is the incidence rate for nonmelanoma skin cancer in the ith age interval, $f_i(D)$ is the relative risk of getting nonmelanoma skin cancer during the ith age interval from exposure to D grams/year of coal tar, and $\ddot{A}_i$ is the width of the ith age interval. The values for c_i were taken from the San Francisco-Oakland subset of the TNCS for 1971-1972 (Scotto et al. 1980), averaged across sexes and adjusted for multiple incidences of nonmelanoma skin cancer per person by dividing by the average number of cancers per patient, 1.12 (Scotto et al. 1974). The choice of the San Francisco-Oakland rates is suggested by the following factors. First, the risk assessment is for California users; the San Francisco-Oakland rates are more indicative of what would be expected in California than any of the other locations (Minneapolis, Iowa, or Dallas). Second, the assumption that rates for San Francisco-Oakland should be acceptable for the entire state is based on these considerations. Although San Francisco-Oakland is in the northern part of the state, which might have slightly lower background incidence rates of skin cancer than southern California, the population of southern California includes more Hispanics. It is known (Scotto et al. 1983) that Hispanics have lower rates of skin cancer than non-Hispanic whites. Moreover, the use of rates based on whites overestimates the rates for blacks, for which skin cancer rates are very low. Therefore, the use of the San Francisco-Oakland rates would overestimate the incidence for a large fraction of Californians. All of these considerations taken together suggest that the San Francisco-Oakland rates for the background rate of nonmelanoma skin cancers are the appropriate choice for the lifetable analysis.

The relative risk of getting nonmelanoma skin cancer during the ith interval, $f_i(D)$ was calculated as

$$f_i = 1 + b \times d,$$

where d is the cumulative exposure at that age (D times the midpoint of the ith interval) and b is the potency factor estimated from the Pittelkow et al. study (1.8×10^{-4}).

The additional risk attributable to exposure to dose rate D is

$$\text{Risk} = \text{LTR}(D) - \text{LTR}(0).$$

If that risk is fixed at a level of interest (one in 100,000, i.e., 10^{-5}), and the background rates and potency factor are estimated as described above, the only parameter that is not determined is *D*. Therefore, *D* can be varied until the risk equals the level of interest. In this case, the lifetable analysis applied to these data gives a lifetime background risk of nonmelanoma skin cancer of 0.10 (Table 3.1-8). Our upper bound dose-response model (i.e., the lifetable calculation using b_u) predicts that coal tar increases the age-specific incidence rates by a factor of $(1+1.8\times10^{-4}\times D)$. When incorporating this information into the lifetable analysis, we assumed that exposure to coal tar occurs at a constant yearly rate throughout life. For *D* equal to slightly more than 0.0108 grams absorbed/year (or 29 µg absorbed/day), the lifetime risk is 1×10^{-5}. Consequently, the 95% lower bound on the dose predicted to result in one extra cancer in 100,000 persons exposed for a lifetime is 29 µg absorbed/day.

Table 3.1-1
Anatomic Distribution of Skin Tumors

Site	Tumor Type*			Total
	No. of Basal Cells	No. of Squamous Cells	No. of Unknown	
Head and neck	12	3	0	15
Upper extremities	0	3	1	4
Trunk	6	1	1	8
Lower extremities	4	0	0	4
Unknown	0	0	1	1
TOTAL	22	7	3	32

Source: Pittelkow et al. (1981)
*Total number of tumors reported in 19 patients with skin cancer.

Table 3.1-2
Median Values for Each Group Possessing Given Characteristic

Characteristic	Median Values *	
	With Skin Cancer	Without Skin Cancer
Years' duration in sun belt after first treatment	14 (18)	15 (58)
Years of home tar use after first treatment	21 (9)	14 (90)
Days of tar treatment (1951-1955)	16 (19)	17 (261)
Days of UV light treatment (1951-1955)	13 (19)	14 (261)
Days of tar treatment (1956-1960)	21 (6)	21 (30)
Days of UV light treatment (1956-1960)	19 (6)	18 (30)

Source: Pittelkow et al. (1981)
* In parenthesis are the number of patients from which these medians are estimated.

Table 3.1-3
Patients in Each Group Possessing Given Characteristic

Characteristic	Percentage of Patients*	
	With Skin Cancer	Without Skin Cancer
Known carcinogenic exposure in occupation	0 (19)	0 (261)
Significant medical history		
Skin cancer	5 (19)	1 (261)
Premalignant skin disease	10 (19)	4 (261)
Other treatments		
Arsenicals	10 (19)	13 (261)
Methotrexate	10 (19)	6 (259)
Ionizing radiation	68 (19)	57 (259)
Coal tar	68 (19)	67 (258)
Ultraviolet light	68 (19)	70 (261)
Resident of sun belt	42 (19)	27 (212)
Brunet hair color at age 20 years	75 (16)	80 (245)
Burns on exposure to sun	31 (16)	29 (124)
Used tar at home	56 (16)	72 (125)
Used tar at home for 50 days or more/year	67 (9)	56 (90)

Source: Pittelkow et al. (1981)

* Percentages are based on patients for whom necessary information was available. Numbers in parentheses indicate number of patients available for comparison.

Table 3.1-4
Estimation of Grams of Coal Tar Used Per Day During a Hospital Stay (G)

Parameter	Mean	Median	Minimum	Maximum
Body Surface Area (m^2)	1.84	1.84	1.4	2.3
Scaling Factor (g/m^2)	0.815	0.815	0.815	0.815
Grams Coal Tar per day per 1% Coal Tar in Ointment (g)	1.5	1.5	1.14	1.87

Table 3.1-5
Days of Treatment During Hospital Stay ($D_{t1,t2}$)

Parameter	Mean	Median	Minimum	Maximum
Days of First Treatment in Hospital Patients who Developed Skin Cancer (D_{t1})	19	17	8	38
Days of First Treatment in Hospital Patients without skin cancer (D_{t1})	19	17	8	38
Days of Second Treatment in Hospital (D_{t2})	22	21	8	38

Table 3.1-6
Distribution Parameters for At-Home Exposure

Parameter	Distribution	Distribution Parameters	Reference
% Coal Tar in Ointment used at home (%CT_h)	Uniform	Minimum=1% Maximum=5%	Dodd (1983) Kanzler and Gorsulowsky (1993)
Fraction of Body Surface Area Treated At-Home (B)	Uniform	Minimum=5% Maximum=25%	Drake et al. (1993)
Days of At-Home Treatment (# 50 days/year) (D_h)	Discrete Uniform	Minimum=10 day Maximum=50 days	Pittelkow et al.(1981)
Days of At-Home Treatment (> 50 days/year) (D_h)	Discrete Uniform	Minimum=51 days Maximum=365 days	Pittelkow et al.(1981)
Years of At-Home Treatment for Patients who developed Skin Cancer (Y_h)	Custom Distribution	Minimum = 4 years Most Likely=20 years Maximum = 27 years	Pittelkow et al. (1981)
Years of At-Home Treatment for Patients without Skin Cancer (Y_h)	Custom Distribution	Minimum = 1 years Most Likely=14 years Maximum = 27 years	Pittelkow et al. (1981)

Table 3.1-7
Estimates of Exposure from At-Home Therapy

Parameter	Mean	Median	Minimum	Maximum
Fraction of Body Surface Area Treated At-Home (B)	0.15	0.15	0.05	0.25
% Coal Tar in Ointment used At-Home ($\%CT_h$)	3	3	1	5
Grams of Coal Tar in a 1% Ointment Required to Cover the Whole Body Treatment Surface Area (grams/day)	1.7	1.7	1.27	2.08
Days of At-Home Treatment (# 50 days/year) (D_t)	30	30	10	50
Days of At-Home Treatment (> 50 days/year) (D_t)	208	208	51	365
Years of At-Home Treatment for Patients who developed Skin Cancer (Y_h)	19	20	4	27
Years of At-Home Treatment for Patients without Skin Cancer (Y)	14	14	1	27

Table 3.1-8
Lifetable Analysis

Age Interval	Average Age	Years of exposure	Mortality per 100,000 Population (1989 US white race both sexes)	Incidence of Skin Cancer per 100,000 Population, White (TNCS, San Francisco Area 1971-72)			Probability of Survival without Skin Cancer		Back ground Probability of Skin Cancer by Year	Probability of Skin Cancer by Year When Exposed to Coal Tar
				Male	Female	Both Sexes (adjusted for multiple cancers)	Unexposed	Exposed		
<1	0.5	0.5	815.5	7.3	3.6	4.87	1.00000	1.00000	0.00005	0.00005
1-4	2.5	2.5	43.2	7.3	3.6	4.87	0.99180	0.99180	0.00019	0.00019
5-9	7	7	21.8	7.3	3.6	4.87	0.98989	0.98989	0.00024	0.00024
10-14	12	12	25.5	7.3	3.6	4.87	0.98857	0.98857	0.00024	0.00024
15-19	17.5	17.5	83.3	7.3	3.6	4.87	0.98707	0.98707	0.00024	0.00024
20-24	22.5	22.5	99.2	7.3	3.6	4.87	0.98272	0.98272	0.00024	0.00024
25-29	27.5	27.5	107.8	37.9	29	29.87	0.97760	0.97760	0.00146	0.00146
30-34	32.5	32.5	128.6	37.9	29	29.87	0.97087	0.97087	0.00145	0.00145
35-39	37.5	37.5	161.7	140.3	87.6	101.74	0.96318	0.96318	0.00490	0.00490
40-44	42.5	42.5	220.2	140.3	87.6	101.74	0.95050	0.95049	0.00484	0.00484
45-49	47.5	47.5	333.8	343.5	233.5	257.59	0.93520	0.93519	0.01204	0.01205
50-54	52.5	52.5	538.1	343.5	233.5	257.59	0.90754	0.90754	0.01169	0.01169
55-59	57.5	57.5	885.2	683.5	382.5	475.89	0.87144	0.87143	0.02074	0.02074
60-64	62.5	62.5	1390.1	683.5	382.5	475.89	0.81213	0.81213	0.01932	0.01933
65-70	67.5	67.5	2080.3	1156	438	711.61	0.73636	0.73635	0.02620	0.02620
									0.10384	0.10385

4.0 Exposure Assessment: Estimation of Intake From a Shampooing Event

4.1 Estimates of Intake to Compare to NSRL Derived Using Human Data

The NSRL estimated using the Pittelkow et al. (1981) data was based on the amount of coal tar ointment absorbed by patients both during their hospital stay and in the years of follow-up home treatment. In comparing exposure to constituents in coal tar from shampooing to this NSRL, the relevant estimate of exposure for the shampoo users is the amount of coal tar absorbed during shampooing. During shampooing, the shampoo is applied, lathered, and rinsed. The time in contact with the scalp for the amount applied is a few minutes. Moreover, there may be some shampoo residue remaining on the scalp after rinsing. The LADD of coal tar constituents absorbed during shampooing and from this residue is the dose that is to be compared to the NSRL derived from the epidemiologically based dose-response analysis.

When estimating the LADD, the manner in which shampoo products are used should be considered. Exposure patterns of these products vary among users. The product may or may not be used daily, depending on the nature of the skin condition of the user. Information on product usage is available from consumer surveys, and this can be used to estimate frequency (days/year) and duration (years). Distributions of product usage can be developed from these data. Moreover, absorbed amounts can be estimated from values published in the literature for excretion of 1-OH-P (see the discussion in Section 3.1.2.1) following shampooing with coal tar-containing shampoos. All of these estimates are variable from one individual to another; representation of that variability by frequency distributions and evaluation of those distributions via Monte Carlo analysis (as was done for the dose-response analysis) constitute the preferred approach for this exposure assessment. Development of the distributions and use of the available data are described in the following sections.

The LADD in µg absorbed/day was calculated using the following equation:

$$LADD_{\mu g/day} = \frac{Conc_{mg/g} \times U_{oz/yr} \times CF_{g/oz} \times Y_{yr} \times F \times 1000\ \mu g/mg}{70\ yr \times 365_{days/yr}}$$

where,

$LADD_{\mu g/day}$	=	Lifetime Absorbed Daily Dose (in µg absorbed/day)
$Conc_{mg/g}$	=	mg of coal tar/gram shampoo

$U_{oz/yr}$	=	ounces of shampoo used/year
$CF_{g/oz}$	=	grams/ounce conversion (28.35)
Y_{yr}	=	number of years of usage
F	=	Fraction of coal tar in applied shampoo that is absorbed
1000	=	µg/mg conversion
70	=	Years in a lifetime
365	=	days/year

Distributions were developed for the parameter ounces of shampoo used per year (*U*), the number of years of usage (*Y*) and the fraction of coal tar in the applied shampoo that is absorbed (*F*) for use in a Monte Carlo analysis to provide a distribution of LADDs. The basis for these distributions is discussed below and presented in detail in Appendix B.

4.1.1 Amount of Coal Tar per Gram of Shampoo ($Conc_{mg/g}$)

The amount of coal tar in shampoos was assumed to range from 0.5% (lowest recommended level in the FDA monograph) to 2.5% (highest concentration in the extra strength formulation for the majority of dandruff shampoos). Estimates of the LADD were calculated for 0.5-2.5%; therefore, the mg of coal tar/gram of shampoo varied from 5 to 25. These values were not part of the distributions developed but rather were used as discrete values such that the estimated LADD for each coal tar formulation with percentage of coal tar varying from 0.5-2.5% was calculated.

4.1.2 Ounces of Shampoo Used Per Year (U)

Two sources of information were available from which to develop estimates of the amount of product used over the course of a year. One source was the AC Nielsen Household Panel market research survey (Nielsen 1999). The second source was a telephone survey conducted by Toppmeyer Research, Inc. (1994).

The Nielsen data were collected in 1997 and 1998 from approximately 40,000 households that scanned their purchases of all UPC coded products each week over a 2-year period. Among the 40,000 households, 3,180 households purchased any coal tar-containing shampoo product over a 2-year period; the average annual amount of shampoo purchased in those households was 7.6 ounces. Data from Nielsen allowed one to calculate the percentage of households with

average annual purchases of coal tar shampoos in half-ounce intervals, i.e., the percentage of households purchasing 0 to ½ ounces per year, the percentage of households purchasing ½ to 1 ounces per year, etc. (Table 4.1.1). Sizes available for purchase ranged from 1 and 3 ounce trial sizes up to 12 ounces. In the category 0 to ½ ounce, ½ ounce was assumed for usage (i.e., one 1-ounce bottle purchased in 2 years).

The second source of usage data (Toppmeyer Research, Inc.1994) was based on spot telephone interviews of residences using Random Digit Dialing to identify a population of shampoo users. A total of 300 current or former users of a specific brand of coal tar-containing shampoo were interviewed and asked questions concerning their shampoo purchases and usage. Forty-nine of the 300 interviewees were current users of the specific brand of coal tar shampoo; of the 251 former users, 122 continued to use some dandruff shampoo, and 111 no longer used a dandruff shampoo (no responses were obtained for 18 of the former users). Persons were asked the size of the bottle most often purchased, the amount of time a bottle of shampoo lasted, the percentage of the bottle personally used, the months per year used, and the number of years of usage. Using these responses, Toppmeyer (1994) reported that the mean total lifetime usage of the specific brand of coal tar-containing shampoo was 570.9 ounces for current coal tar shampoo users and 114.3 ounces for former users. The mean total lifetime usage for all 300 responders was 189.1 ounces. In order to compare these figures with the average yearly purchase data from Nielsen (1999), responder-specific data obtained directly from Toppmeyer Research, Inc. (e.g., bottles purchased per year, length of time a bottle lasts, etc.) were used to estimate that the current and former users interviewed reported an average use of 37.0 ounces per year.

While both sets of usage data provide estimates of shampoo usage, the data from the Nielsen (1999) study are considered more reliable. The Toppmeyer (1994) survey was a telephone survey designed to estimate market penetration, not to estimate coal tar usage, and was for a single brand of coal tar-containing shampoo only. The telephone interview approach used by Toppmeyer (1994) is much less reliable than the product scanning approach of the Nielsen (1999) study. It has associated with it numerous recall problems and relies on the use of guesses concerning purchase and use patterns. Such problems lead to overestimates of the frequency of heavier use: those individuals who used more of the shampoo are more likely to recall and report that use (especially for the 84% of the interviewees who were no longer using the shampoo in question). Moreover, the selection of the 300 interviewees would have been biased by the same

recall problems: individuals who used less of the shampoo would tend to be excluded from the interview process altogether.

These concerns about the Toppmeyer data on amount of shampoo use are confirmed by empirical evidence. Some of the reported use patterns were clearly not accurate. For example, three individuals stated that they had used the coal tar-containing shampoo in question for more than 45 years; at the time of the interview, the product had been on the market for approximately 20 years. Furthermore, if the average ounces/year calculations using the Toppmeyer (1994) data are extrapolated to total use, using the "household penetration" data for the shampoo in question from Nielsen (1999), and then used to project annual retail sales of that product, the result is an estimate that is more than 4 times the actual retail sales reported by the manufacturer. In comparison, the same projection based on the average ounces/year from Nielsen (1999) resulted in a projected annual sales that was within 13% of the actual sales figures.

For these reasons, and also because the sample size of the Nielsen (1999) study is so much greater and therefore provides more stable estimates, the distribution describing variability in average annual ounces of coal tar-containing shampoo used (parameter *U*) is based on the data supplied by Nielsen (1999). That distribution is a custom-designed, piecewise uniform distribution. Each piece has a length of ½ ounce (from 0 to ½, from ½ to 1, from 1 to 1½, etc.) and the total probability associated with each piece is equal to the fraction of the Nielsen households that purchased any coal tar-containing shampoos that had an annual purchase amount within that range (Table 4.1-1; Appendix B). The average annual use of coal tar-containing shampoos ranges up to 136 ounces/year.

The use of the Nielsen (1999) data to represent average annual use would tend to overestimate exposure for at least two reasons. First, the data are for purchases, not use, *per se.* If any of the purchased shampoo is not used (e.g., spilled or discarded before the entire bottle is used), then use (exposure) will have been overestimated. Second, the Nielsen (1999) data reflect household purchases, not purchases by a single individual. So, to the extent that coal tar-containing shampoos are shared by more than one household member, exposure will again be overestimated.

Finally, the use of the Nielsen (1999) data will overestimate the amount of variability in average use. The parameter of interest for the exposure assessment is average yearly use, when considering the entire history of shampoo use. The averages calculated from Nielsen (1999) are

based on 2 years of purchasing. The use of a shorter period of observation leads to the well-known phenomenon commonly referred to as "regression towards the mean" whereby the extremes of a distribution of observations tend towards the center of that distribution given additional (longer) observation. The effect of this overestimation of variability is that extreme input values (and therefore extremes of the outputs calculated via Monte Carlo analysis) will be over-represented.

The distribution parameters used in the Monte Carlo analysis for ounces of shampoo used per year are given in Table 4.1-2. The simulation results (for 200,000 iterations) yielded a mean and median ounces/year equal to 7.4 and 4.2, respectively, a minimum of 0.5 ounces/year, and a maximum of 136 ounces/year (Table 4.1-3).

4.1.3 Years of Usage (Y)

In the Toppmeyer (1994) survey, the number of years of product usage was one of the questions posed. The number of years of use of a particular brand of coal tar-containing shampoo reported by Toppmeyer (1994) had a mean of 4.2 years for total responders and 8.08 years for current users. Fifty-nine percent of the current product users reported using the product for 5 years or less, while 10% used the product for 6-9 years, 16% for 10-15 years and 14% for 16 or more years. However, because these responders were replying from memory, there was some evidence of recall bias. For example, three individuals recalled using the product for more than 45 years, when at the time, the product had been on the market for about 20 years.

Rather than years of usage, the number of years a person suffered from psoriasis or protracted dandruff of the scalp was considered a more reliable surrogate for duration of use. Therefore, the years that an individual suffered from such a skin condition were determined for each of the responders: the mean number of years that responders were said to have suffered from dandruff, seborrhea or psoriasis was 10.8 for total users (current and former) and 11.76 for current users. The number of years of suffering from one of the skin conditions ranged from 1 to 21 or more (Table 4.1-4).

The Toppmeyer (1994) data represent snap-shots in time of the history of potential coal tar-containing shampoo use for individuals of various ages. Of interest for this exposure assessment is the years of use over the course of a 70-year lifespan. In order to estimate such lifetime use, the data from Toppmeyer (1994), which included age groupings for the responders,

were used in a regression analysis. The years of suffering from a skin condition were regressed against age (actually the middle value of the age category reported for each individual); age was the independent variable and years of suffering was the dependent variable. The estimated linear relationship [years of suffering = 0.26*age] had an R^2 value of 0.58 (accounting for nearly 60% of the variation in the dependent variable) and predicted that at age 70, the average years of suffering from one of the conditions associated with use of coal tar-containing shampoo would be 18.3.

In order to derive a distribution for years of use at age 70, we used the standard deviation of years of suffering from among those who were in the 65-74 and 75+ age categories, i.e., those who had at least 70 years of potential shampoo use, as the estimate of the standard deviation for the distribution. The value of that standard deviation was 14.5. There was apparent skewness in the observations: more than half of them were less than the predicted means but there was a relatively long "tail" above those means with a few extreme values. All these considerations together suggested that a lognormal distribution with a mean of 18.3 years and a standard deviation of 14.5 years (truncated at 70 years) would be an appropriate descriptor of variability in years of use during a 70-year lifespan.

For comparison purposes, the predictions based on the regression of the Toppmeyer (1994) data were examined relative to the experiences of cohorts who were known to suffer from skin conditions requiring coal tar therapy. Pittelkow et al. (1981), as discussed in detail in Section 3.1, reported median values for years of home treatment for their cohort of severely affected psoriasis patients. Considered all together, that cohort had a median for years of home treatment of roughly 14 or less. The regression-based distribution used in this exposure assessment had a median of 14 years. Menter and Cram (1983) reported the duration of coal tar usage for a cohort from psoriasis day care centers to range from greater than 5 years to greater than 40 years (Table 4.1-4). Among that cohort, 55% used coal tar for 10 or more years, 25% used coal tar for 20 or more years, and 6% used coal tar for 30 or more years. The regression-based distribution used in this exposure assessment estimates about 70%, 30%, and 15% for 10 or more, 20 or more, and 30 or more years of use, respectively, all greater than the percentages reported by Menter and Cram (1983).

It is apparent that the distribution used in this exposure assessment for years of use of coal tar-containing shampoo includes a higher percentage of long-term users than were observed in

the clinical cohorts of severely affect psoriatics. On the one hand, this is appropriate if one considers that the full course of treatment for the clinical cohorts may not have been captured at the time of publication of the cited studies. On the other hand, the observations from these studies represented an extensive follow-up time (Pittelkow et al. 1981) or years of clinical experience (Menter and Cram 1983). Moreover, the observations in these studies were based on severely affected individuals, those who presumably would require more and longer treatment than the typical shampoo user who is self-treating dandruff or some other less severe scalp condition.

Finally, note that in this exposure assessment based on the regression of the Toppmeyer (1994) data, the surrogate used for years of use of a coal tar-containing shampoo is years of suffering from a skin condition. The use of that surrogate is very likely to overestimate the actual years of use. First, a treatment (shampoo use) may not be initiated as soon as the condition appears, nor would it necessarily be maintained over the entire course of the condition. Second, the options for treatment include other, noncoal tar shampoos that would likely be used for at least some of the time. In fact, more than 40% of the Toppmeyer (1994) interviewees were using dandruff shampoos other than the coal tar-containing brand in question.

Considered all together, the distribution defined for the parameter *Y*, years of use of a coal tar-containing shampoo, appears to be reasonable. If it errs, then it is likely to overestimate the number of years of use. Note also that, because the distribution of years of use includes values as large as 70 years, the exposure assessment is accounting for exposures that may have begun in childhood and continued throughout the lifetime of the individual.

4.1.4 Fraction of the Applied Amount that Is Absorbed (F)

The dose of coal tar, and its corresponding 95% lower bound, associated with the target risk level (1×10^{-5}) were estimated based on the amount of coal tar absorbed into the skin of psoriasis patients. Consequently, the relevant dose metric for estimation of the LADD for shampoo users is the amount of coal tar in shampoo that is absorbed. Just as in the case of coal tar ointment use, the fraction of the applied amount that is absorbed should be estimated from studies where excretion of the pyrene metabolite 1-OH-P is observed following exposure. In this case, the exposure is to coal tar-containing shampoo. Differences in absorption would be expected between exposure of psoriasis patients to coal tar in an ointment versus exposure to coal

tar in a shampoo by the average shampoo user, due to matrix effects, as well as duration of contact. Data were available from a study conducted by van Schooten et al. (1994), in which urinary 1-OH-P excretion was measured in volunteers following exposure to a coal tar shampoo (van Schooten et al. 1994) similar to those available in the State of California. The use of different studies for the fraction of coal tar absorbed for the psoriasis patients and shampoo users accounts for the expected differences in absorption that can be attributed to the different matrices and duration of exposure.

In the study conducted by van Schooten et al. (1994), eleven volunteers (two smokers, four males and seven females) washed their hair with 20 grams of a coal tar-containing shampoo for a total of one minute (two, 30-second applications). The shampoo used, Resden Forte, is comparable to pharmaceutical grade coal tar-containing shampoos sold in the United States that contain 2.5 % or greater coal tar. Two individuals of the same age (nonsmokers, one male and one female) served as controls, using a shampoo that did not contain coal tar. The coal tar-containing shampoo used had a total PAH level of 2840 mg/kg, which included approximately 10% pyrene (285 mg pyrene/kg shampoo) and 2% BaP (56 mg/kg shampoo). Urinary excretion data for 1-OH-P was measured in the urine the day prior to shampooing, and then two urine samples in a 24-hour period were collected on days one and two after shampooing. A mean value of 34.0 nmol 1-OH-P was recovered in the urine in 48 hours post-shampooing, with the majority of that excreted in the first 24 hours. A total of 4.9 nmol 1-OH-P was excreted by the unexposed controls over that same 2-day period.

To generalize this data to the population of hypothetical shampoo users, the same approach as used in the dose-response assessment was applied here as well. It was assumed that the distribution of 1-OH-P excretion from this defined exposure was lognormal. The mean of that distribution was set equal to the difference between the excretion observed after exposure (34 nmol) and that observed without exposure (4.9 nmol). The standard deviation for this distribution was set equal to the largest standard deviation of 1-OH-P excretion observed for any one of the urine collection periods, 10.04 nmol. The largest standard deviation was selected because the distribution being estimated here is intended to be a generalization to the larger population of hypothetical shampoo users. To exclude unrealistically large or small values, the lognormal distribution was truncated at 49.1 nmol and 0.5 nmol, respectively. A value sampled

from this distribution was converted to µmols by dividing by 1000. This distribution provides estimates of the amount of 1-OH-P that would be excreted (Table 4.1-2).

Given the distribution for amount of 1-OH-P excreted, the distribution for fraction of pyrene absorbed (as a surrogate for fraction of coal tar absorbed) is estimated from the amount of pyrene applied (which was fixed in the van Schooten study at 28.2 µmol) and the same distribution for the conversion factor, C_f, as used for the individuals who absorbed pyrene from an ointment (Santella et al., 1994; Section 3.1.2.1):

$$F = \frac{P_e / P_a}{C_f}$$

where

P_e	=	amount of pyrene excreted as the 1-OH-P metabolite (µmol)
P_a	=	amount of pyrene applied in shampoo (µmol)
C_f	=	a conversion factor specifying the fraction of absorbed pyrene that is metabolized to and excreted as 1-OH-P (unitless).

The resulting distribution for *F* defines the variation from one individual to another with respect to fraction of coal tar absorbed (Table 4.1-3).

4.2 Estimates of Dermal Dose Following Coal Tar Shampooing

A Monte Carlo analysis based on the equation to estimate the LADD was conducted using the distributions described in the preceding sections; the data output from that analysis are found in Appendix E. Under Proposition 65 in the State of California, determination of the need to warn is based on exposure to the typical product users. The median values from the distributions of LADDs were selected as being representative of the typical user.

Compared to the median user, any other user is equally likely to have had a greater exposure or to have had a lesser exposure. In contrast, the mean value could be (and commonly is) at a point in the distribution such that it is less likely to have an exposure greater than the mean than it is to have an exposure less than the mean. Figure 4-1 displays the median and mean values for the LADD from this exposure assessment, using the results of a 2.5% coal-tar shampoo as a case in point (see Appendix D). Note that the median is much more central with respect to the LADD values. USEPA (USEPA 1992) recognizes the median as an appropriate

basis for characterizing the "central tendency" of a distribution. Furthermore, in the context of this exposure assessment the median, not the mean, is the appropriate representation of typical use. Note that the output distribution from this exposure assessment represents variability over individual users, as a consequence of the fact that the input distributions represent variability over individuals. Therefore, each point on that distribution represents the possible exposure of any one shampoo user. The median represents the center ("typical" location) of those individuals.

Specifically, the output is not intended to represent different values of a variable that must be "aggregated" to get a relevant measure and, therefore, stands in stark contrast to a situation where the different values from the output distribution must be added together or averaged to characterize an individual's exposure. Such a case might arise in a situation where the output variable represented distinct contributions making up an individual's total exposure. Say, for example, that the output distribution represents yearly intakes of some contaminant. Then, assuming that a random individual would have yearly intakes randomly selected from the underlying distribution, over a 70-year lifetime an individual would have a total exposure equal to the sum of randomly selected values from that distribution, or an average yearly exposure that could reasonably be represented by the average (mean) value from the distribution. In this instance, the mean is a better indicator of typical exposure, just because the output parameter still needs to be "aggregated" to get to a measure typical of any one individual. In contrast, the output distribution of the exposure assessment for shampoo users is already specific to individuals who are exposed. Thus, it is not appropriate to characterize the typical user by the mean of the output distribution; the aggregation that gets one to the individual exposures has already been accomplished (through the definition of the parameters Y and U, which are lifetime average values) so that averaging at the end is inappropriate. The median is the value that typifies the experience of any one individual.

By way of further explanation of the appropriateness of the median and the inappropriateness of the mean in the context of this exposure assessment, consider this example. Suppose that by observation, it is known that LADDs for some compound in some population can only take the following values, in equal proportions: 1, 2, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 11, and 99.5. That is, for all individuals in the population, they are equally likely to have any one of the 13 values listed, but only one of those 13 values. The median value is 6; the mean value is 13.

Here the typical LADD is better characterized by the median than by the mean: typically, a random selection of an individual will result in a LADD closer to the median than to the mean, which is greater than all but one of the observed values!

Table 4.1-5 presents the median values (i.e., typical user exposures) for coal tar-containing shampoos with a percent coal tar ranging from 0.5% up to 2.5%. The estimated median LADDs ranged from approximately 1 µg/day at 0.5% to 5 µg/day for the 2.5% product. These values are all less than the NSRL derived from the data reported by Pittelkow et al. (1981), as discussed in greater detail below (Section 5.1).

Table 4.1-1
AC Nielsen Household Panel

Total Coal Tar (Products) (3,180)	
Ounces Purchased	Percent of Buyers
0 - 5	56.74
5 - 10	22.88
10 -15	8.13
15 - 20	5.73
20 - 25	2.77
25 - 30	1.15
30 - 35	0.84
35 - 40	0.58
40 - 45	0.33
45 - 50	0.05
50 - 55	0.32
55 - 60	0.29
60 - 70	0.04
70 - 76	0.03
123 -136	0.12

Source: AC Nielsen Household Panel (1999).

Table 4.1-2
Other Assumptions in Monte Carlo Analysis of Exposure through Shampoo Use

Parameter	Distribution	Distribution Parameters	Reference
Number of Years of Usage	Discretized Lognormal	Mean = 18.37 Std =14.47 Maximum = 70	Toppmeyer (1994)
1-OH-P Excreted by Body	Lognormal	Minimum = 0.5 nmol Mean=29.06 nmol Std=10.04	Van Schooten et al. (1994)
% of pyrene metabolized	Uniform	Minimum = 3% Maximum = 81%	Van Rooij et al. (1993a, 1993b, 1995), Van Rooij (1995), Jongeneelen and Bos (1990) Singh et al. (1995)

Table 4.1-3
Results in Monte Carlo Analysis of Exposure through Shampoo Use

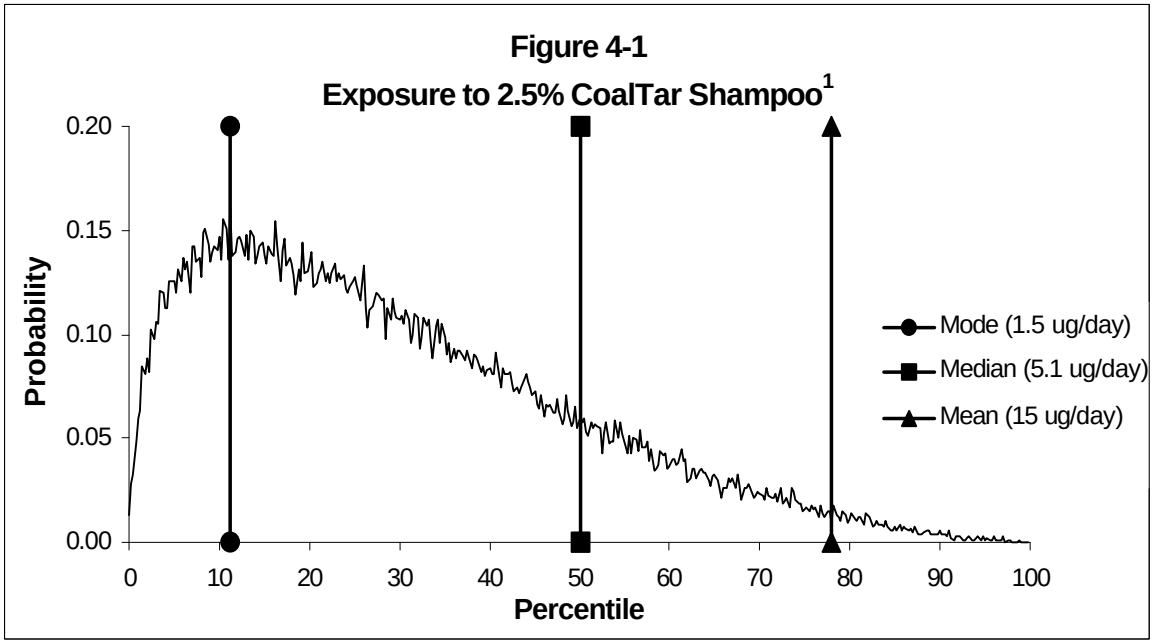
Parameter	Mean	Median	Minimum	Maximum
Ounces of Shampoo Used per year (U)	7.43	4.2	0	135.9
Number of Years of Usage (Y)	18	14	1	70
% Coal Tar Applied That is Absorbed (F)	0.42	0.24	0.03	5.7

Table 4.1-4
Data Related to Years of Shampoo Usage

Study	Years of Usage	Number of Individuals	Fraction in Category
Toppmeyer (Years suffered from symptoms)	1	45	0.15
	2	33	0.11
	3	27	0.09
	4	12	0.04
	5	21	0.07
	6-9	27	0.09
	10-15	57	0.19
	16-20	36	0.12
	>20	42	0.14
		300	
Menter and Cram	5	81	0.45
	10	57	0.32
	20	34	0.19
	30	6	0.03
	40	2	0.01
		180	

Table 4.1-5
Estimates of Lifetime Average Daily Dose (µg/day) from Exposure to Coal Tar in Shampoo from Monte Carlo Analysis of Exposure through Shampoo Use

% Coal Tar in Shampoo	Median (50th percentile) (µg/day)
2.5	5.1
2.0	4.1
1.8	3.7
1.6	3.3
1.4	2.9
1.2	2.5
1.0	1.7
0.5	1.0



NOTE: The results shown here are those from Appendix D representing 300,000 Monte Carlo simulations of possible LADD values.

5.0 Risk Characterization

In the Risk Characterization step in the risk assessment process, estimates of exposure (determined in the Exposure Assessment step) are compared to estimates of either a cancer potency factor or to a dose associated with a target risk level (developed in the Hazard Assessment and Dose-Response Assessment steps) to provide an estimate of the risk to the target population for the exposure conditions identified. Identification and characterization of the assumptions and uncertainty inherent in the assessment are part of the Risk Characterization, as well as a discussion of the impact on interpretation of the estimated risks for the designated population.

The exposure scenario of interest is exposure to coal tar constituents found in pharmaceutical grade coal tar-containing shampoos used at home in the treatment of psoriasis, seborrhea, and other dermatosis of the scalp. The target tissue of interest is the skin. This assessment was conducted to determine if the concentrations of coal tar in coal tar-containing, anti-dandruff shampoos would result in an estimated exposure, defined as the amount of coal tar constituents that is absorbed into the skin following shampooing events (expressed as a LADD in µg/day) for a typical shampoo user, that exceeds the NSRL, defined as the specific, conservative (health protective) lower bound on absorbed dose associated with a one in 100,000 risk.

Neither bioassay data nor epidemiological studies were available in which a coal tar-containing shampoo was the test article. Rather, data from psoriasis patients treated with coal tar ointments were available and were reviewed (Section 2.0). The data found to be most suitable for estimation of a NSRL were the epidemiological data related to exposure to coal tar ointments among psoriatics (Pittelkow et al. 1981). However, the overall conclusion of this clinical data was that the use of pharmaceutical grade coal tar to treat psoriasis did not result in an increased risk of nonmelanoma skin cancer. Therefore, use of these negative data results in upper bound estimates of risk. Moreover, the NSRL value itself is a conservative estimate, because it is based on a potency value (the 95th percentile of all the potencies derived from the Monte Carlo analysis) that falls above the range of estimates from the Monte Carlo analysis that USEPA defines as “high-end estimates” (USEPA 1992).

5.1 Comparison to NSRL

A dose-response analysis was based on epidemiological data for a group of patients treated with coal tar for psoriasis at the Mayo Clinic (Pittelkow et al. 1981). Using exposure information published about the cohort as well as information in general about the treatment modalities at the Mayo Clinic and other psoriasis clinics using coal tar ointment therapeutically at that time, the amounts of total coal tar used by each patient over their course of treatment were estimated. Using Monte Carlo analysis, the average amount of coal tar absorbed from ointment used both during the initial hospital treatment and subsequent at-home treatment was estimated to be 252 grams. When combined with uncertainties associated with the observed and the expected numbers of nonmelanoma skin cancers, an upper bound potency was determined (1.8×10^{-4} per gram absorbed). A lifetable analysis conducted with this upper bound potency provided a conservative estimate of the LADD associated with the target risk level, which for the purposes of this analysis was one extra cancer in 100,000. That dose was 29 µg absorbed coal tar/day.

Estimates of the LADD, again using Monte Carol analyses, provided estimates of exposure to typical coal tar-containing shampoo users for coal tar content ranging from 0.5% to 2.5%. These values ranged from approximately 1 µg of absorbed coal tar/day for the 0.5% coal tar shampoo up to 5 µg absorbed coal tar/day for the 2.5% shampoo, all of which are lower than the target dose of 29 µg/day (Appendix D). Stated another way, the ratio of the estimated exposure from long-term use of coal tar-containing shampoos to the NSRL associated with a risk of 1×10^{-5} is less than one.

5.2 Discussion of Uncertainty

5.2.1 Uncertainties in the Dose-Response Model Using Human Data

As in any risk assessment, there are uncertainties associated with the estimates of risk discussed above. One strength of the analysis as presented here is that uncertainties associated with quantitative estimation [e.g., of the average cumulative exposure for the members of the Pittelkow et al. (1981) cohort, or of the underlying probability of nonmelanoma skin cancer in that cohort] have been incorporated into the analysis methodology. As a result, the analysis results have been able to provide an estimate of the risk of using coal tar-containing shampoo that appropriately reflects the level of confidence that can be associated with the input parameters. In fact, the analysis has provided an estimate of the risk that is conservative in a definable sense:

given the data now available and the treatment of the quantitative uncertainties associated with estimation of the underlying realities (e.g., estimation of the uncertainty about the true probability of response based on assumption of an underlying Poisson process for the observed number of cases), the conservative estimate of risk is one that underestimates the true risk with a defined and small probability (i.e., with probability less than 0.05). Conversely, the conservative estimate of dose associated with a target risk is one that overestimates the true dose associated with that risk with a defined and small probability (0.05).

5.2.1.1 Qualitative Uncertainties

The above statements are conditional on other factors, several of which are assumptions made in the course of the analysis. They may be considered to be unquantified uncertainties. In this section are listed some of the more salient assumptions or uncertainties associated with this risk assessment.

The first "uncertainty" is that associated with the use of the Pittelkow et al. (1981) cohort as the basis for the risk assessment. The relevant uncertainties in this regard concern the appropriateness of cohort as a sample from the general population. If it can be considered to provide a fair representation of what would generally be expected under the specific exposure conditions under study for coal tar-containing shampoo usage, then it must be considered an appropriate basis for the risk assessment. [Indeed, it could be argued that if the likelihood of response for the patients in the cohort is thought to be *greater* than that of the general public, then the cohort still provides an adequate basis for a risk assessment, yielding even more conservative estimates, but conservative estimates that are not directly quantifiable.]

Whether psoriasis skin is a good model for shampooer's skin with regard to skin tumor formation is an important issue and one which we have studied. As discussed in Section 2.2.2.2, psoriatic skin and normal skin compare favorably with regard to absorption of constituents in coal tar (Santella et al. 1994; Van Schooten et al. 1994) and other agents used to treat psoriasis (Schaefer et al. 1977;Wang et al. 1987; Wester et al. 1983). Metabolic capability, as indicated by AHH basal and induced levels, microsomal epoxide hydrolase activity, and BaP metabolism, were similar in persons with psoriasis and in nonpsoriatic individuals (Bickers et al. 1984; Bickers and Kappas 1978). Moreover, DNA adduct levels (Phillips et al. 1990; Schoket et al. 1990), DNA repair rates (Dybgahl et al. 1999), and induction of ODC levels with tape stripping

(Arnold et al. 1990), and a lack of induction of ODC levels with pharmaceutical grade coal tar (Arnold et al. 1993) were comparable in both populations. The general consensus from studies in which the incidence of skin cancer among psoriatics was compared to the incidence rate in the general population was that psoriasis did not confer either protection or increased sensitivity (Alderson and Clark 1983; Bhate et al. 1993; Stern et al. 1985). An increased incidence of skin cancer in psoriasis patients was associated with other forms of treatment, in particular PUVA, but not with coal tar therapy alone. Indeed, the incidence of skin cancer in persons treated for nonpsoriasis skin conditions with the standard Goeckerman regimen (coal tar and UV-B light) was not increased above that in a reference population (Jemec and Østerlind 1994; Maughan et al. 1980). Note also that persons using coal tar-containing shampoos are using these products for medicinal reasons to treat psoriasis or other skin condition of the scalp. Therefore, potential coal tar- containing shampoo users may have more in common with a psoriatic population than they would with other members of the "normal" population. It is expected that use of this population to derive a NSRL for coal tar in anti-dandruff shampoos is appropriate and will be representative of coal tar-containing shampoo users.

Another potential "uncertainty" associated with use of the Pittelkow et al. (1981) study relates to its sample size, or its "power." "Power" is a statistical term relating to the likelihood of rejecting a hypothesis. In particular, "the power of a test against the alternative q is the probability of rejecting H when q is true" (Bickel and Doksum 1977), where q specifies some particular state different from that specified by H, the null hypothesis. Power is analytically equal to one minus the probability of type II error (accepting the null hypothesis, H, when, in fact, H is not true).

There are three things to note about this definition of power. First, the concept of power is only applicable in the context of hypothesis testing; as implied by the definition given above, one considers power when one wants to test the conditions implicit in the statement of H, and the goal is the determination of whether or not one can reject that null hypothesis.

Secondly, power is specific to a particular test procedure. So, not only must one be testing a hypothesis, one must specify the particular test that will be used to test that hypothesis. Different possible tests have different powers against any given alternative.

Thirdly, even when a specific test statistic is chosen, power is not a single fixed value. The power of a test varies as a function of the alternative, q. The more "different" the alternative is from the null hypothesis, the greater the power should be (for any reasonable test). If the null hypothesis is that $q=0$, in some example hypothesis-testing context, then the probability of rejecting H (i.e., the power) should be greater when $q=1$ than when $q=0.1$.

In the context of the risk assessment for coal tar-containing shampoos, the concept of power does not apply. The analysis does not propose a particular test of some null hypothesis. In fact, the analysis is not a hypothesis testing exercise at all. The Pittelkow et al.(1981) study is not intended here to be the basis of a hypothesis test, and, therefore, statistical considerations other than power are the appropriate basis for evaluation of that study.

Traditionally, confidence limits on an estimate (in this case, of the relative risk) would be that appropriate basis. In this analysis, an extension of the confidence limit concept was used; we were not interested in just a single point reflecting one level of confidence. Rather we needed, and calculated, the probabilities associated with the entire range of possible values for relative risk, and used them in the Monte Carlo procedure described above. In any case, the adequacy or contribution of a study in a risk assessment is not measured by "power," but rather by the distribution of possible values for an underlying parameter (e.g., relative risk or probability of response) estimated using that study. As noted above (Section 3.0), the use of a smaller study for estimation (as opposed to hypothesis testing) leads to more conservative (health protective) risk estimates, all else being equal. The size of the Pittelkow et al. (1981) study does not detract from this analysis and should not be considered to be associated with additional "uncertainty."

Three other uncertainties associated with the Pittelkow et al. (1981) study have been determined. The first relates to the fact that nonmelanoma skin cancers are not generally fatal and that, therefore, an individual may develop more than one such tumor. The TNCS rates used by Pittelkow et al. (1981) to estimate expected numbers of persons with skin cancer need to be adjusted for tumor multiplicity to derive correct rates on a per person basis. Since TNCS reports an average of 1.12 skin tumors per person, division by 1.12 would correct the reported rates. It is assumed that Pittelkow et al. (1981) made this adjustment when calculating expected numbers of skin cancers. We have assumed that they did do so because the TNCS is explicit about their rates and Pittelkow et al. (1981) repeatedly state that they have derived the expected number of *people*

with skin cancer, not the expected number of cancers. However, if the adjustment was not made by Pittelkow et al. (1981), then the expected numbers are too high by a factor of 1.12. So, for instance, the observed (best estimate of) relative risk would be (19/260)/(23.8/260) = 0.80 rather than 0.71. The 95% confidence limit on that relative risk would be (27.9/260)/(23.8/260) = 1.17 rather than 1.05.

Incidentally, the lifetable analysis corrected for the fact that nonmelanoma skin cancers are not generally fatal by defining the "survival" rates used in the lifetable in terms of survival *without* nonmelanoma skin cancer. In that way, no individual contributes more than one such tumor to the determination of (background or exposed) number of persons with skin cancer.

For this assessment, focused on California users of pharmaceutical grade coal-tar containing shampoos, it was determined that for the lifetable analysis the background rate of nonmelanoma skin cancers for the San Francisco-Oakland area was the appropriate choice. However, it should be noted that the use of national rates, rather than California-specific rates, does not significantly change the estimated NSRL. Use of national rates of nonmelanoma skin cancers in the lifetable analysis decreases the NSRL slightly from 29 µg/day to 27 µg/day.

Another uncertainty associated with the use of the Pittelkow et al. (1981) study concerns the possibility that not all of the skin cancers that the cohort developed had been reported in that study. The possibility of over- or under-reporting of skin cancers during the period of follow-up is handled quantitatively and discussed in the next section. The possibility of skin cancers occurring after the follow-up period does exist. Note, however, that at that same time when the number of observed skin cancers might have increased, the number of skin cancers that would be expected would also have increased. Therefore, it is not clear if the estimate of the relative risk would go up or go down (or remain unchanged) with additional follow-up. The follow-up time for the cohort as reported in the 1981 report had reached as much as 27 years, so this may not be a source of great concern.

The final uncertainty associated with the use of the Pittelkow et al. (1981) cohort relates to the level of exposure of these individuals. The analysis described in Section 3.1 accounts for exposures to coal tar that occurred at or after the initiation of the Goeckerman treatment at the Mayo Clinic. It also quantitatively accounts for uncertainties about those exposure levels. However, it is known that some of the cohort members had been exposed to coal tars prior to the Goeckerman therapy (Table 3.1-3). In addition, the application of the ointment described by

Muller and Perry (1984) as typical of the therapy at the Mayo Clinic specifically stated that the head was not treated. They go on to describe a Nivea oil-based scalp treatment that was used at least by 1967. It is likely that members of the Pittelkow et al. (1981) cohort also used some treatment containing coal tar for their scalp, although such use is not reflected in the exposure estimates developed above for this cohort. Because of prior coal tar exposure and lack of reporting of scalp treatment, it is clear that the exposure level for the Pittelkow et al. (1981) cohort has been underestimated. The extent of that underestimation is uncertain, but its effect has been to overestimate the potency and, therefore, underestimate the NSRL, i.e., to yield a NSRL that is too small.

Another factor that contributes to the underestimation of total exposure for this cohort is the fact that the assumed maximum number of days over which the cohort members received in-hospital coal tar treatment was 38 for each of the two time periods considered (1951-1955 and 1956-1960). That upper limit is based on a reasonable bound for a single hospitalization (Menter and Cram 1983) but if more than one hospitalization occurred in either of those time periods, then it is likely that the total number of days of in-hospital treatment could have exceeded 38 days. Again, this potential underestimation of total exposure would lead to an overestimation of the potency and a smaller NSRL.

An uncertainty in the estimates of exposure of coal tar to persons in the Pittelkow et al. (1981) cohort was that the absorbed dose was back-calculated from data for 1-OH-P in the urine. In this case, 1-OH-P levels were measured in the urine of volunteers who were being treated with a coal tar ointment by the same protocol as that reported by Pittelkow et al. (1981) (Santella et al. 1994) and the presumed distribution for the parameter C_f, defining the proportion of absorbed pyrene that is metabolized to and excreted as 1-OH-P, was used to convert the urinary levels to an estimated absorbed dose. This is uncertain for three reasons, each of which would affect estimates for the psoriasis cohort. The first is the use of 1-OH-P, the major metabolite of pyrene, as a biomarker of exposure to PAHs. The underlying assumption in the use of these data is that the ratio of pyrene to total PAHs remains relatively constant in a specific coal tar mixture[7]

[7]As indicated in Section 2.0, the term coal tar can refer to a wide variety of mixtures of PAH-containing materials all derived from the same source material, coal, by the fractional distillation of that source material. The chemical composition of the specific coal tar mixtures formed by the distillation of coal, such as pharmaceutical grade coal tar, creosote, and coal tar pitch, is dependent on the temperature range at which the specific mixture was collected. Constituents in coal will distill or fractionate at different temperatures. Consequently, creosote, which is

(Jongeneelen and Bos 1990). This approach has been very useful in assessing changes in exposure to PAHs for different job categories or occupations, as well as in assessing the effectiveness of various industrial hygiene practices in reducing exposure to PAHs. However, to use urinary 1-OH-P levels to quantitatively assess dermal absorption of other PAHs requires the assumption that other PAHs have the same absorption characteristics (rate and percentage) as pyrene. While urinary 1-OH-P is a useful biomarker of pyrene exposure, when used as a biomarker to quantify exposure for other specific PAHs, the absorbed amount of lower molecular weight PAHs (e.g., fluorene, phenanthrene) will be underestimated, and that of higher molecular weight PAHs [e.g., BaP, benzo(k)fluoranthene] will be overestimated (Dankovic et al. 1989; Jongeneelen et al. 1986; Roy et al. 1987; Van Rooij et al. 1995). Based on a review of these studies, the data indicate that use of pyrene as a surrogate for absorption of the PAHs of interest, namely the higher molecular weight PAHs that are considered to be the carcinogenic constituents, would result in an overestimate of absorption for BaP by at least a factor of 3, based on a 24-hour exposure, and a factor of 7 for about a 4-hour exposure. Other PAHs would be overestimated by a much greater amount (Dankovic et al. 1989; Roy et al. 1987; Van Rooij et al. 1995). Consequently, the amount of absorption may be overestimated. However, 1-OH-P data was relied upon for estimating the absorbed dose in both the dose-response and exposure assessments. Therefore, if absorption is overestimated for the dose-response assessment, it is likely overestimated similarly for the exposure. It is expected that although the use of pyrene data to estimate absorption of constituents from coal tar may be an overestimate for constituents other than pyrene, the relative absorption between pyrene and other coal tar constituents following ointment or shampoo usage will be similar.

The second uncertainty in using urinary 1-OH-P levels to estimate the absorption fraction is that data from several studies report widely varying values for C_f. This probably represents a combination of interindividual variation as well as experimental error (uncertainty with respect to the true value of this parameter due to differences in experimental techniques and practices). Because we have, in essence, associated all of the variability in this parameter with interindividual variation, we most likely have not underestimated that variation. Consequently,

---

collected at temperatures in the 300 to 400 degree range, will have a difference constituent profile and toxicological properties than that of pharmaceutical grade coal tar collected at temperatures ranging from 900 to 1100 degrees. However, for a given coal tar mixture, the relative amounts of pyrene to other PAHs is assumed to be similar.

using this wide range in a Monte Carlo analysis should not result in an underestimate of the range of pyrene absorption in the hypothetical shampoo users. Note also that uncertainty and variability estimates for C_f will affect the dose-response analysis (estimates of potency and the NSRL) and the exposure assessment (estimates of typical use exposure levels) in the same way, so the relative difference between the typical exposure and NSRL should be maintained, however the distribution of C_f is defined.

The third reason has already been discussed with regard to uncertainties in estimating exposure to the Pittelkow cohort, and this is the uncertainty in the distribution of values used to provide estimates of absorbed dose from urinary excretion data. In a reanalysis in which an alternative distribution for this parameter was not done because changing the distribution for this parameter had little effect on the estimate of the NSRL, it was expected that a similar, small change would result and in the same direction as that for the NSRL.

Because of some of the uncertainties associated with using urinary metabolite data to estimate the absorbed dose in the skin, another dose metric, total DNA adduct levels in the skin, was investigated as a measure of the absorbed fraction. As discussed previously (Section 2.4.2.1), studies were conducted by Godschalk et al. (1998) in which a group of eczema patients was topically treated with coal tar ointment for 7 to 33 days. The focus of this study was to examine the applicability of DNA adducts in white blood cell populations as a measure of dermal exposure to PAHs. No clear dose-response relationship was observed between exposure and total adduct level or urinary metabolites and corresponding adduct levels. The authors concluded that the sensitivity of DNA adducts in white blood cell subpopulations as a measure of exposure is limited, with measurements of urinary PAH metabolites being more sensitive. While DNA adducts may be a biomarker of exposure, there appears to be no correlation of total adducts with exposure or with response. DNA adducts are readily formed in individuals exposed to coal tar ointment (Godschalk et al. 1998); however, no significant increase in the incidence of skin cancer has been identified in cohorts of patients with various skin disorders who have been exposed to large doses of coal tar ointment (Pittelkow et al. 1981; Jones et al. 1981; Maughan et al. 1980). In addition, a method for relating DNA adducts to absorbed dose does not exist; therefore, this dose metric could not be used to estimate an absorbed dose for the development of a NSRL. Therefore, although DNA adducts were considered qualitatively as another possible

dose metric, the data were not available to use this quantitatively to estimate an absorbed dose following coal tar ointment or shampoo application.

In addition to those uncertainties associated with the use of the Pittelkow et al. (1981) study are uncertainties or assumptions inherent in the quantitative analysis. Two of those assumptions are listed here.

A linear relationship between total exposure and relative risk has been assumed. That is not the only relationship that could have been used. However, with a single treated group [Pittelkow et al. (1981)], the options for estimating alternative relationships are limited: only a single unknown parameter can be estimated (the potency factor, *b*, was the unknown parameter estimated from the Pittelkow et al. data). The choice of a linear relationship is a standard of cancer risk assessment methodology and is recognized as a conservative choice in cancer risk assessment practice. Therefore, while the use of the linear relationship for relative risk is an uncertainty, it is not likely to contribute to an underestimation of low-dose risk.

Finally, once the relationship between relative risk and total exposure had been determined for the Pittelkow et al. (1981) cohort, that relationship had to be "extrapolated" to the general population via the lifetable calculations. To do so, it was assumed that the estimated relationship held for all ages. That is, the relative risk was assumed to be constant, for any given value of total exposure. This is a standard assumption used in most risk assessments.

As stated, all of the above-mentioned uncertainties are present in the analysis performed here. They are typical of the uncertainties inherent to most, if not all, risk assessments. They are not necessarily quantifiable in the sense of being able to yield confidence limit statements; the quantifiable uncertainties associated with estimation of input parameters have been considered in the derivation of the conservative bounds on risk or dose and in the following section. In general, the qualitative uncertainties discussed above suggest that, if anything, the dose-response treatment of the available data will have led to conservative estimates of the NSRL, i.e., to overestimate the potential health consequences of exposure to coal tar in shampoos.

5.2.1.2 Quantitative Uncertainties

Each of the uncertainty parameters contributing to the calculation of a patient's total exposure to coal tar was assigned a particular distribution to describe its uncertainty. Moreover, distributions representing the uncertainty related to the probabilities of response with and without

exposure (parameters underlying the calculation of the relative risk) were also defined. This is the essence of the Monte Carlo procedure. Nevertheless, the exact nature of those distributions (e.g., whether a triangular distribution is a better descriptor of the uncertainty than a truncated normal distribution) is most often not known. Therefore, the assumptions made concerning those distributions must be recognized as uncertainties of the analysis. It can be stated that the choices for the distributions were made in such a way as to best reflect the information that was available, including information from ancillary sources (e.g., data from other patients treated with the Goeckerman therapy at the Mayo Clinic or elsewhere). Nevertheless the quantitative impact of those choices can be ascertained; those impacts are summarized here.

Because of the numerous parameters requiring uncertainty distributions, the following approach was used to identify those that were in greatest need of a quantitative evaluation. Each of the parameters was cross-classified with respect to two factors: first, a subjective assessment of the relative level of uncertainty associated with knowledge about the parameter and, second, the inherent sensitivity of the dose-response result (the potency estimate) to the value of the parameter. The results of that cross-classification are shown in Table 5.1-1.

The relative ranking of uncertainties associated with the parameters was based on factors such as the amount of information that was available to characterize the uncertainty or variability in the parameters. In addition, the range of possible values for a parameter could also determine the relative ranking: a parameter that was more constrained would be ranked as being less uncertain than a parameter that was less constrained, all else being equal. As an example, the days of home use per year for those using the ointments at home for less than 50 days/year (parameter $D_{h<50}$ in Table 5.1-1) was considered less uncertain than the parameter $D_{h>50}$ defining the number of days per year of home use among those treating 50 or more days/year.

The sensitivity of the potency estimates to the parameters was defined analytically as follows. First, the potency was calculated when all parameters were set equal to their average values. Then, one at a time, the parameter values were increased by 10% and the percent change in the potency estimate was determined. Thus, a 10% change in the potency, for the 10% change in a parameter value, would indicate a proportional effect; any change in potency of less than 10% would indicate a less than proportional effect. The grouping used in Table 5.1-1 considered the parameters inducing less than a 7.5% change to constitute the lowest sensitivity group, those

inducing a 7.5% to 10% change to constitute the middle group, and those inducing greater than a 10% change to constitute the highest sensitivity group.[8]

Based on the results of the cross-classification of the parameters with respect to uncertainty and sensitivity, the following parameters were chosen for additional examination: $\%CT_h$, B, $D_{h>50}$, $P(d)$, $P(0)$, and the three parameters defining the fraction of coal tar absorbed, C_f, P_a, and P_e. These were all the parameters that had both an uncertainty grade of 2 or greater and a sensitivity grade of 2 or greater. For each of these parameters, an alternative distribution describing its uncertainty was selected, and, one parameter at a time,[9] a complete Monte Carlo analysis was redone using the alternative distribution. The impact on the NSRL estimate was determined.

Table 5.1-2 shows the results of the uncertainty analysis. In general, the quantitative impact of varying the assumptions about these parameter is not great, and, moreover, the selected changes tend to increase the NSRL estimate. In no case did the alternative assumptions change the NSRL in such a way that it was less than the estimated exposure level of a typical shampooer using a shampoo with coal tar concentration equal to or less than 2.5%.

For the parameter $\%CT_h$, the percent of coal tar in the ointment used at home, the alternative distribution shifted the concentration to slightly higher concentrations than those considered in the main analysis (because the endpoints of the distribution are 2 and 10 rather than 1 and 5), while at the same time making the endpoints of the alternative range much less frequent than the midpoint (i.e., because the distribution is triangular rather than uniform). The shift to slightly higher concentrations is suggested by the data from a variety of sources describing home use of coal tar ointments (Dodd 1993; Kanzler and Gorsulowsky 1993; Leong 1990; Silverman

---

[8]A slightly different treatment was considered for the parameter P_u, summarizing the treatment of those individuals for whom no information about home treatment modality was available. The approach considered best was to assign those individuals randomly for each iteration of the Monte Carlo, where the assignment probabilities were equal to the proportions observed among those with known home treatment modalities. For the base case of the sensitivity analysis, the one specific allocation closest to that suggested by those proportions was chosen (as an "average" assignment). For the test of sensitivity, another specific allocation was chosen, i.e., that closest to an equal split of the individuals in question to the three home treatment modalities (none, less than 50 days/year, or more than 50 days/year). As indicated in Table 5.1-1, the potency estimate was relatively insensitive to these choices.

[9]The one exception was for the three parameters defining the fraction of coal tar absorbed. Because they are related in such a way that biologically impossible values must be ruled out, the alternatives for these parameters were considered together. That is, there was one Monte Carlo analysis for these three parameters combined.

et al. 1995). The range of ointments described in those sources ranged up to 25%, but Kanzler and Gorsulowsky (1993), in particular, stated that relatively few of the patients tolerated more than about 5% coal tar. Thus, the alternative distribution represents a desire to include use of some possibly greater concentrations (in our distribution, only up to 10%) but to make the likelihood of use of the higher concentrations less than the likelihood of a more moderate but clinically effective concentration (with 6% being the most likely concentration in our alternative distribution). The use of this alternative resulted in a change in the NSRL from 29 to 54 µg/day.

The distribution for the proportion of the body surface area treated at home (the parameter *B*) was altered to reflect experiences of clinicians who suggested that 25% as an upper bound might be too low. Suggested alternatives were based on 30% as either a maximum or an average. The alternative triangular distribution, having both a maximum of 30% and a most likely value of 30%, addresses both of those suggestions, to different extents: the maximum is 30% but the mean of that distribution is 21.7%. This alternative was believed to be more representative of the data than a distribution with a mean of 30%, but it still allows for some values greater than 25%. The NSRL would change from 29 to 39 µg/day if this alternative distribution for *B* was used as the basis for NSRL derivation.

The number of days of use per year among those using ointments at home more than 50 days/year was obviously constrained by the boundaries 51 and 365 days. Pittelkow et al. (1981) provided no more information relevant to definition of a distribution for this parameter, so the main analysis assumed a uniform distribution between those boundaries. However, as an alternative, if it is assumed that the most likely frequency of use is about 3 days/week (or about 150 days/year) then a triangular distribution can be used. This alternative also shifts the median days of use per year from 208 to 181. While other alternative distributions could be chosen (e.g., one for which the values corresponding to particular numbers of days/week – 52 for 1 day/week, 104 for 2 days/week, 156 for 3 days/week, etc. – were given more weight than other values between 51 and 365), the chosen alternative gives a sufficient indication of the impact of uncertainty about this parameter. The resulting minor change in the NSRL is from 29 to 26 µg/day.

The normal distribution assumed for the expected number of skin cancers in the main analysis was centered at the average value of the four expected numbers reported in Pittelkow

et al. (1981). This is believed to be the best value to use to describe the center of the distribution of each individual's contribution to the expected number. One could, as an alternative, use that average as the most likely value of a triangular distribution, having endpoints equal to the smallest and the largest of the four reported values (Pittelkow et al. 1981), which were also used to truncate the normal distribution of the main analysis. When that is done, the mean of the expected numbers changes from 26.6 to 30.4. More significantly, the 95th percentile of the distribution of potency estimates becomes negative, indicating that even when conservative choices are made (e.g., a high-end estimate of the potency is estimated) there is no indication that there is an increased risk attributable to these coal tar exposures. Stated another way, the NSRL is infinite because no dose level will produce a risk as large as one in 100,000.

The issue of the observed number of skin cancers and estimates of the probability of response, *P(d)*, is a complicated one. If one assumes that the reported number of nonmelanoma skin cancer cases, 19, is correct, then the only uncertainty about *P(d)* is that associated with its estimation from a finite sample (i.e., it is purely statistical and it is handled appropriately as described in Section 3.1.3). However, there may be some uncertainty about whether or not 19 is the correct value for the observed number of cases. On the one hand, some individuals in the cohort followed by Pittelkow et al. (1981) had died by the time the follow-up occurred. Responses to questions about the presence or absence of nonmelanoma skin cancer were supplied by relatives, in those instances, and it is possible that some existing cases were not reported because the relatives forgot or were unaware of the existence of the cancer.

On the other hand, there are at least four factors mitigating the potential under-reporting. First, these patients were being examined for a skin condition, psoriasis, that made them, and by extension their relatives, acutely aware of health problems associated with the skin, including cancer, which in and of itself is not something likely to be forgotten. The relatives would be even less likely to be unaware of health-related skin problems, to the extent that psoriasis conditions tend to run in families. Under-reporting would at least be lessened by the heightened awareness associated with the follow-up of a clinically treated cohort.

The potential for under-reporting by family members was investigated. No data were found that specifically addressed nonmelanoma skin cancer; however, studies for melanoma and other cancers were identified. One study by Aitken et al. (1996) evaluated the reporting bias of

relatives of patients with melanoma and found a 40% false-positive rate, i.e., over-reporting. Other skin cancers may have been confused with melanoma, accounting for some of the false positives.[10]

Second, the fact that these patients were being followed for skin conditions entails a surveillance bias. Because of the increased frequency and diligence of medical examinations for this cohort, relative to the general public, more skin cancers would tend to be discovered in the Pittelkow et al. (1981) cohort. While this is an issue that can be viewed as relating to the values of the expected numbers of cancers in this cohort [i.e., that they would be even greater than those determined in the TNCS (Scotto et al. 1974), which were based on routine visits to doctors among the general public], it is considered here as a mitigator of possible under-reporting. In essence, because the number of cancers reported by this cohort is expected to be greater than otherwise would be the case because of surveillance bias, if any under-reporting has occurred, then that just serves to bring the observed number back in line with the expected rates that have been used in this analysis.

Note that in the survey reported by Scotto et al. (1974), "a special effort was made to encourage all dermatologists, pathologists, radiotherapists, and other physicians who see and treat skin cancer to participate" (Scotto et al. 1974, p. 1334). While this special effort makes it more likely that all the reports available to doctors have been obtained, it does not negate the effect of surveillance bias. This is so because no special effort was made to get ordinary people to consult a physician, i.e., there was no greater chance that these general populations were examined for skin cancer (in contrast to the members of the Pittelkow et al. cohort). Indeed Scotto et al. (1974) state that under-reporting "may not be ruled out yet" (p. 1337), despite the special effort to get all doctors to participate in their study.

A third and related consideration is that the patients comprising the Pittelkow et al. (1981) cohort had had therapies for their skin conditions that are known to increase the risk of skin cancer. These include exposure to arsenicals, methotrexate, and ionizing radiation (Table

---

[10]While not as relevant to the case of skin cancer, Aitken et al. (1995) and Breuer et al. (1993) assessed the validity of family reporting of colorectal cancer and breast cancer. In both of these cases, over-reporting of cases by family members was the norm. While the literature does not contain any studies directly relevant to over-reporting or under-reporting of nonmelanoma skin cancer, the literature sources cited do not suggest that under-reporting by family members is likely.

3.1-3). So, as discussed in the preceding paragraph, it is likely that the expected numbers based on the general population would be too low (fewer members of the general population have had such exposures) to be strictly applicable to this cohort. Any possible under-reporting of skin cancers again serves to bring the observed and expected numbers back in line with one another.

Finally, data in Pittelkow et al. (1981) directly suggest that *over*-reporting of nonmelanoma skin cancers may have occurred. Pittelkow et al. (1981) obtained histologic specimens for 14 of the 22 individuals who originally reported a cutaneous malignant neoplasm. Among those 14 individuals, two were determined not to have had malignant neoplasms at all. This is a 14% over-reporting. Because there were still eight patients who did not have specimens reviewed, it is possible, and the data directly from this cohort would support the contention, that at least one of the eight other individuals reporting nonmelanoma skin cancers did not, in fact, have nonmelanoma skin cancer.

If one considers all these factors together, then one must acknowledge that the number of observed skin cancers could equally well have been over-reported as under-reported. Treating this uncertainty quantitatively required an assumption of some alternative number of observed cases; both an increase of about 10% (two additional cases) and a decrease of about 10% (two fewer cases) were used as alternatives. A change of plus-or-minus 10% was chosen as a reasonable basis for this uncertainty investigation based on the factors discussed in the previous paragraphs. While the possibility of under-reporting by relatives exists, it is counter-balanced by the fact that family members would be more aware of such cancers than normal, because of the focus on skin conditions for these cohort members. Moreover, relative to the expected numbers used in this analysis, the observed incidence could already be considered to be too high, even without the consideration of under-reporting. Finally, direct information from Pittelkow et al. (1981) suggests over-reporting. The 10% difference, in either direction, assumed for the alternative analyses is an amalgam of these considerations.

The alternative assumptions required the estimation of the relative likelihoods of the possible true response rates, given the alternative observed numbers (17 or 21), in the same manner as described above for the reported value of 19 (Section 3.1.3). As in the main analysis, this was done assuming the observed number of cases is the result of sampling from a Poisson random variable. If the alternative number of 21 is used, the NSRL decreases from 29 to 10

µg/day, the latter value still being about a factor of 2 greater than the LADD associated with typical use of a shampoo with 2.5% coal tar. When the alternative value of 17 is used, the NSRL becomes infinite. As in the case of the alternative distribution for the expected number of cancers, this indicates that even a conservative approach to risk estimation fails to find any finite dose that causes a one in 100,000 risk. The uncertainty about the number of skin cancers in the Pittelkow et al. (1981) cohort does not affect the conclusion that the typical use scenario yields exposures less than the NSRL.

Finally, as a group, alternative distributions were assumed for the three parameters that define the fraction of coal tar that is absorbed from the applied ointment. These parameters were treated together because they jointly define the absorption fraction and the values had to be generated so that biologically impossible values (e.g., absorption fractions greater than 1) were avoided. As noted in Section 3.1.2.1, the data supporting the distributions for these parameters do not come from the Pittelkow et al. (1981) cohort directly. They were based on data from other psoriatics treated with a Goeckerman-like therapy (Santella et al. 1994) and on data related to conversion of absorbed pyrene to the 1-OH-P metabolite. So, rather than change the center of the distributions derived from those sources, which we believe to be appropriate for use with psoriasis patients, we just assumed a different shape for those distributions. Hence, the uniform distribution for C_f was changed to a triangular distribution, giving more weight to values in the center of the range from 0.03 to 0.81. The lognormal distributions for P_a and P_e were instead assumed to be normal (truncating on the lower end at zero), with the same means and standard deviations. The net effect of these changes was to increase the NSRL from 29 to 44 µg/day. Once again, there was no indication that the uncertainty about the distributions of these parameters would affect the conclusion that the typical exposure for a shampooer is less than the NSRL.

5.2.1.3 Conclusion of Uncertainty Analysis of Human-Based NSRL

The potential impact of the uncertainties listed here should be considered in the Risk Characterization and may legitimately influence the decision-making process. It is worth noting that, although much has been included here about uncertainty, many of the uncertainties confronting most risk assessments, primarily those associated with extrapolation from animal test

species to humans, are absent from this risk assessment. Because this assessment was based on a relatively well-controlled epidemiological study, the relative level of confidence for this assessment as compared to other risk assessment should be high, especially with respect to the conclusions regarding the fact that typical LADDs are uniformly less than the NSRL.

This is particularly the case because, considered all together, the qualitative as well as the quantitative uncertainties addressed here strongly suggest that the NSRL value, 29 µg/day, to which exposure estimates were compared is unlikely to be too low. This conclusion represents the synthesis of the qualitative uncertainties discussed above (in particular the fact that the level of exposure for this cohort is undoubtedly underestimated) as well as the trend of the quantitative uncertainties and their analytical impact on the NSRL estimates. While it is clear that the NSRL can change with some alternative assumptions (ones that were not considered to be optimal ones), this is only to be expected for any assessment when such a thorough uncertainty analysis is completed. No matter how the quantitative calculations were altered, the NARL was consistently greater than the dose associated with typical use, as defined in Section 4.1. Because the qualitative uncertainties would either tend to increase the NSRL or decrease the typical shampoo user's LADD, one can be confident that that typically does not exceed a dose that would be associated with a target risk of one in 100,000.

5.2.2 Uncertainties in Estimating Exposure from the Use of Coal Tar-Containing Shampoos

Estimation of the LADD for shampoo users required four pieces of information: concentration of the coal tar in the shampoo, ounces/year of shampoo used, years of shampoo usage, and fraction of coal tar in shampoo that is absorbed. The concentration of coal tar in the shampoo was assumed to cover a range of concentrations from 0.5% to 2.5% of the shampoo. These are based on actual concentrations found in shampoo. Estimation of a LADD for a product with a concentration outside of this range is a simple linear extrapolation, given the assumptions that have been used.

The ounces per year of coal tar shampoo use and the years of usage were derived from survey data (Nielsen 1999; Toppmeyer 1994) with supporting information from published literature sources (Menter and Cram 1983). While the population size used to estimate the

ounces of shampoo used per year (Nielsen 1999) was relatively large (almost 3200 households), the data for the number of years of usage were from smaller populations. Moreover, the years of use parameter had to be extrapolated to a full 70-year lifespan using a regression approach. This introduced some uncertainty into the selection of the distribution to represent variability in years of use.

As with estimates of exposure for members of the Pittelkow cohort, a major uncertainty in the estimates of exposure to persons using coal tar-containing shampoos was that the absorbed dose was back-calculated from data for 1-OH-P in the urine (see Section 5.2.1.1). In this case, 1-OH-P levels were measured in the urine of volunteers who used a coal tar-containing shampoo (van Schooten et al. 1994), rather than psoriasis patients treated with a coal tar ointment (Santella et al. 1994). Absorbed fraction in the shampooers was back-calculated from data for 1-OH-P in the urine in the same manner as it was calculated for the psoriasis patients, resulting in the same uncertainties, as discussed in Section 5.2.1.1.

In addition, data on multiple steps are needed to estimate absorbed dose using 1-OH-P data, including the amount of pyrene applied to the skin and how much of the amount of pyrene absorbed is metabolized to 1-OH-P and excreted in the urine. No one study quantified each of these components, and a wide range of values was derived based on the literature. Nevertheless, use of this wide range in a Monte Carlo analysis should not result in an underestimate of exposure to the shampoo users.

Some differences in absorption would be expected between different formulations of shampoos; however, the data needed to quantify these differences was not identified. In van Schooten et al. (1994), relied upon to estimate absorption of constituents in pharmaceutical grade coal tar from a shampoo matrix, a commercially available brand of a coal-tar containing shampoo was used for the analysis. Although the shampoo matrix used in different formulations may result in slight increases or decreases in absorption of constituents in pharmaceutical grade coal tar, it is unlikely that absorption for other formulations would be underestimated, because van Schooten et al. (1994) relied upon a brand that contained one of the higher concentrations of PAHs in the brands evaluated. Therefore, these results from this study were assumed to be representative of all commercially available formulations of coal-tar containing shampoos.

Since psoriasis may occur in children and children may be users of coal tar-containing shampoos, the possibility of an increased lifetime risk for children was considered an uncertainty and was evaluated as follows. Steps were taken in the analysis of the NSRL to include in the lifetable ages less than one year and included four new groups: <1, 1 to 4, 5 to 9, and 10 to 14 years. The uncertainty that exposure as a child would engender a higher extra lifetime skin cancer risk considered two components: a potential increase in the sensitivity of child's skin to constituents in coal tar and a potential increase in the total lifetime exposure.

With regard to the potential for increased sensitivity of infant or child skin, the following evidence was considered. Basal AHH activity levels are comparable in neonatal foreskin and adult epidermal skin sections (0.1 and 0.06 pmoles 3-OH BaP/min/mg protein, respectively) and responded similarly (about a 3-fold increase in AHH activity) following induction with BA (Alvares et al. 1972; Bickers et al. 1984; Levin et al. 1972). Neither human fetal skin nor adult skin xenografts developed tumors following application of DMBA with TPA promotion (Graem 1986). Production of SCCs was noted in 2/47 human neonatal xenografts following DMBA and UV-B treatment but not in 13 adult xenografts similarly treated (Soballe et al. 1996) suggestive but not clear evidence of an enhanced response in neonatal skin (a Fisher' Exact test fails to reject the null hypothesis of no difference, $p = 0.61$). DNA repair rates are comparable in human fetal skin and adult skin with adult skin removing 50% of UV-induced pyrimidine dimers over a 16-hour period and fetal skin removing 65% of pyrimidine dimers over a 24-hour period (Gibson-D'Ambrosio et al. 1983; Taichman and Setlow 1979). The review of the available literature provided no clear evidence of an increased sensitivity of infant or child skin compared to adult skin.

With regard for the potential for increased lifetime exposure, the following was considered. Estimates of exposure would be higher in children if the absorption into the skin of children was greater than that of adult skin. Singer et al. (1971) determined that in mammalian species the stratum corneum, which is considered to be the barrier layer to absorption, was fully developed at birth and at birth the ultrastructure of the stratum corneum was indistinguishable from that of the adult. Their data in rats and guinea pigs indicated that the development of the stratum corneum begins in the last quarter of gestation and is completed just before term, which would explain why transient differences in absorption were noted in premature infants compared

to adults. Wester et al. (1977) found that dermal absorption of testosterone in newborn and adult Rhesus monkeys was not significantly different. Further, Cunico et al. (1977) reported no differences in two parameters indicative of skin penetration rates when 22 term infants were compared with 30 adults. Consequently, no separate analysis to incorporate differences in absorption across ages was conducted. Rather, the estimates of LADD from shampooing assumed that the amount of shampoo used by children and adults was the same (i.e., differences in surface area that may translate into less shampoo use by children was not considered) and the years of usage was expanded to range from less than 1 to 70 years.

Recall that for both of the parameters defining total exposure to coal tar-containing shampoo (ounces used per year and years of use) the estimates derived were expected to overestimate the actual values (Sections 4.2 and 4.3). For ounces used per year, this is because the surrogate measure (household purchases) does not account for spillage or other less-than total use of a purchased bottle of shampoo, nor does it account for the fact that there may be more than one user per household. Years of use would be overestimated because the surrogate measure was years of suffering from a scalp condition requiring medicated shampoos. As discussed, a coal tar-containing shampoo is not the only option for home treatment nor would any treatment be applied for the entire duration of the condition. Again, the result is overestimation of the parameter values. For both of these parameters, their overestimation leads to overestimation of LADD. Typical use, therefore, is overestimated and the margin of safety between typical use and the NSRL should be even greater than the results of this analysis suggest.

5.3 Conclusions

The purpose of this project was, in the context of a quantitative risk assessment, to compare estimates of the LADD of coal tar constituents that can be expected following use of pharmaceutical grade coal tar-containing shampoos according to the user scenarios defined by the distributions of the concentration of coal tar in shampoos, the amount of coal tar-containing shampoo used (on a yearly basis), the number of years of usage, and the amount of the effective dose, i.e., the residue fraction that remains in contact with the skin. The NSRL estimated based on epidemiology data was larger than the corresponding LADD for all concentrations of coal tar-

containing shampoos considered. Estimates of exposure to persons regularly using coal tar-containing shampoos were below the 95% lower bound on dose at the target risk level of one in 100,000.

Table 5.1-1
Cross-Classification of Parameters Used in the Estimation of Potency [1]

Sensitivity [2]	1	2	3
1	G, G_h, P_u	D_1, D_2, $D_{h<50}$	
2		% CT_h, B	$D_{h>50}$
3		P(d), P(0)	C_f, P_a, P_e

1 Subjective, relative ranking of uncertainty concerning parameters. A rank of 1 is lowest uncertainty.

2 Analytical, relative ranking of sensitivity of potency estimate to value of parameter.
1 less than 7.5% sensitivity
2 7.5% to 10% sensitivity
3 greater than 10% sensitivity

Sensitivity equals percent change in potency for a 10% change in average parameter value (all other parameters remaining fixed at their average values).

NOTE: Parameters as defined in Sections 3.1.2 and 3.1.3, with the following additions:

P_u Assignment of individuals with unknown home treatment characteristics

$D_{h<50}$ Days of home treatment per year, for those with less than 50 days/year of such treatment

$D_{h>50}$ Days of home treatment per year, for those with more than 50 days/year of such treatment

P(d) Probability of response in the cohort (based on observed number of skin cancer cases).

P(0) Probability of response in absence of exposure (based on expected number of skin cancer cases).

Table 5.1-2
Results of Uncertainty Analysis of Selected Parameters

Parameter	Preferred Distribution	Alternative Distribution	Alternative NSRL (µg/day)
% CT_h	U (1,5)	T (2, 6, 10)	54
B	U (5, 25)	T (5, 30, 30)	39
D	U (51, 365)	T (51, 150, 365)	26
P(0) [Expected No.]	N (26.6, 3.5)	T (15.5, 26.6, 49.2)	4
P(d) [Observed No.]	Poisson w/19 observed	Poisson w/21 observed Poisson w/17 observed	10 4
Fraction Absorbed: C_f P_a P_e	 U (0.03, 0.81) LN (6.8, 11.5) LN (98.1, 50)	 T (0.03, 0.42, 0.81) N (6.8, 11.5) N (98.1, 50)	44

NOTES: U(x,y) is uniform with lower bound *x* and upper bound *y*.
T(x,y,z) is triangular with minimum *x*, most likely value *y*, and maximum *z*.
N(x,y) is normal with mean *x* and standard deviation *y*.
LN(x,y) is lognormal with mean *x* and standard deviation *y*.
The Poisson distribution associated with the observed number is actually the distribution of possible response rates, assuming that the observed number arises from a Poisson distribution (see text).
Infinite NSRLs arise because the 95th percentile on potency is negative, i.e., even for a high end estimate, no risk is apparent.

6.0 References

Agency for Toxic Substances and Disease Registry (ATSDR). 1996. Toxicological Profile for Wood Creosote, Coal Tar Creosote, Coal Tar, Coal Tar Pitch, and Coal Tar Pitch Volatiles. ATSDR, Atlanta, Georgia. August 1996.

Agency for Toxic Substances and Disease Registry (ATSDR). 1995. Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs). ATSDR, Atlanta, Georgia. August 1995.

Aitken J, Bayed C, Ward M, Siskin V, McLennan R. 1995. How accurate is self-reported family history of colorectal cancer? Am J Epidemiol 141:863-871.

Aitken JF, Youl P, Green A, McLennan R, Martin NG. 1996. Accuracy of case-reported family history of melanoma in Queensland, Australia. Melanoma Res 6:313-317.

Aizu E, Nakadate T, Yamamoto S, Kato R. 1986. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-mediated epidermal ornithine decarboxylase induction and skin tumor promotion by new lipoxygenase inhibitors lacking protein kinase C inhibitory effects. Carcinogenesis 7:1809-1812.

Alderson MR, Clarke JA. 1983. Cancer incidence in patients with psoriasis. Br J Cancer 47:857-859.

Alexandrov K, Rojas M, Bourgeois Y, Chouroulinkov I. 1983. The persistence of benzo(a)pyrene diol-epoxide deoxyguanosine adduct in mouse skin and its disappearance in rat skin. Carcinogenesis 4:1655-1657.

Alvares AP, Kappas A, Levin W, Conney AH. 1972. Inducibility of benzo(a)pyrene hydroxylase in human skin by polycyclic hydrocarbons. Clin Pharmacol Ther 14:30-40.

American Industrial Health Council (AIHC). 1994. Exposure Factors Sourcebook. AIHC, Washington, DC.

Andrews FJ, Halliday GM, Narkowicz CK, Muller HK. 1991a. Indomethacin inhibits the chemical carcinogen benzo(a)pyrene but not dimethylbenz(a)anthracene from altering Langerhans cell distribution and morphology. Br J Dermatol 124:29-36.

Andrews FJ, Halliday GM, Muller HK. 1991b. A role for prostaglandins in the suppression of cutaneous cellular immunity and tumour development in benzo(a)pyrene – but not dimethylbenz(a)anthracene-treated mice. Clin Exp Immunol 85:9-13.

Angel JM, DiGiovanni J. 1999. Genetics of skin tumor promotion. Prog Exp Tumor Res 35:143-157.

Angel JM, Beltran L, Minda K, Rupp T, DiGiovanni J. 1997. Association of a murine chromosome 9 locus (Ps11) with susceptibility to mouse skin tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate. Mol Carcinog 20:162-167.

Annamalai R, Vasantha M, Umaselvam M, Ashraf MP. 1981. Multiple keratoacanthoma and squamous cell carcinoma in psoriasis. Int J Dermatol 20:6096-6097.

Arnold WP, van Hooijdonk CA, Mier PD, van de Kerkhof PC. 1990. Induction of ornithine decarboxylase following sellotape stripping in normal and psoriatic skin. Br J Dermatol 125:581-585.

Arnold WP, Litsen AM, van de Kerkhof PC, Mier PD. 1991. Cyclo-oxygenase products do not participate in the induction of ornithine decarboxylase in human epidermis. Br J Dermatol 125:577-579.

Arnold WP, Pennings BHJ, van de Kerkhof PCM. 1992. The induction of ornithine decarboxylase in human epidermis is independent of lipoxygenase and cyclo-oxygenase pathways. J Dermatol 19:461-464.

Arnold WP, Glade CP, Mier PD, van de Kerkhof PCM. 1993. Effects of sphingosine, isoquinoline and tannic acid on the human tape-stripping model and the psoriatic lesion. Skin Pharmacol 6:193-199.

Ashendel CL, Boutwell R. 1979. Prostaglandin E and F levels in mouse epidermis are increased by tumor-promoting phorbol esters. Biochem Biophy Res Commun 90:623-627.

Atillasoy ES, Elenitsas R, Sauter ER, Soballe PW, Herlyn M. 1997. UVB induction of epithelial tumors in human skin using a RAT-1 mouse xenograft model. J Invest Dermatol 109:704-709.

Ball CB. 1885. Tar cancer. Irish J Med Sci 80:318-321.

Banks-Schlegel SP, Howley PM. 1983. Differentiation of human epidermal cells transformed by SV40. J Cell Biol 96:330-337.

Bell J. 1876. Paraffin epithelioma of the scrotum. Edinburgh Med J (as cited in Butlin 1892, Lecture III).

Berry D, Bracken W, Slaga T, et al. 1977. Benzo(a)pyrene metabolism in mouse epidermis. Analysis by high pressure liquid chromatography and DNA binding. Chem Biol Interact 18:129-142.

Bertalanffy FD. 1964. Tritiated thymidine versus colchicine technique in the study of cell population. Cytodynamics, Lab Invest 13:871-886.

Bertalanffy FD, Lau C. 1962. Cell renewal. Intl Rev Cytol 13:357.

Berton TR, Mitchell DL, Fischer SM, Locniskar MF. 1997. Epidermal proliferation but not the quantity of DNA photodamage is correlated with UV-induced mouse skin carcinogenesis. J Invest Dermatol 109:340-347.

Bhate SM, Sharpe GR, Marks JM, Shuster S, Ross WM. 1993. Prevalence of skin and other cancers in patients with psoriasis. Clin Exp Dermatol 18:401-404.

Bickel PJ, Doksum KA 1977. Mathematical Statistics: Basic Ideas and Selected Topics. Holden-Day, Inc., San Francisco. p. 167.

Bickers D. 1983. Drug, carcinogen and steroid hormone metabolism in the skin. In: Goldsmith L, ed. Biochemistry and Physiology of the Skin. Oxford University Press, New York. pp. 1169-1186.

Bickers DR, Kappas A. 1978. Human skin aryl hydrocarbon hydroxylase. Induction by coal tar. J Clin Invest 68:1061-1068.

Bickers DR, Mukhtar H, Dutta-Choudhury T, Marcelo CL, Voorhees JJ. 1984. Aryl hydrocarbon hydroxylase, epoxide hydrolase, and benzo(a)pyrene metabolism in human epidermis: comparative studies in normal subjects and patients with psoriasis. J Invest Dermatol 83:51-56.

Bloom W, Fawcett DW. 1975. A Textbook of Histology. W.B. Saunders Co., Philadelphia, PA. pp. 563-597 (as cited in Urano et al. 1995).

Boerrigter M, Wei YU, Vijg J. 1995. Induction and repair of benzo(a)pyrene-DNA adducts in C57BL/6 and BALB/c mice: association with aging and longevity. Mech Ageing Develop 82:31-50.

Boffetta P, Jourenkova N, Gustavsson P. 1997. Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes Control 8:444-472.

Bohr VA, Smith CA, Okumoto DS, Hanawalt PC. 1985. DNA repair in an active gene: removal of pyrimidine dimers from the *DHFR* gene of CHO cells is much more efficient than in the genome overall. Cell 40:359-369.

Bourin MC, Delescluse C, Furstenberger G, Marks F, Schweizer J, Klein-Szanto A, Prunieras M. 1982. Effect of phorbol esters on guinea pig skin *in vivo*. Carcinogenesis 3:671-676.

Boutwell R. 1976. The biochemistry of preneoplasia in mouse skin. Cancer Res 36:2631-2635.

Boutwell R. 1978. Biochemical mechanism of tumor promotion. In: Slaga T, Sivak A, Boutwell R, eds. Carcinogenesis, A Comprehensive Survey, Vol 2. Mechanisms of Tumor Promotion and Carcinogenesis. Raven Press, New York. pp. 49-58.

Boutwell RK, Bosch DK. 1958. The carcinogenicity of creosote oil: its role in the induction of skin tumors in mice. Cancer Res 18:1171-1175.

Bowden GT, Hohneck G, Fusenig NE. 1977. DNA excision repair in ultraviolet-irradiated normal and malignantly transformed mouse epidermal cell cultures. Cancer Res 37:1611-1617.

Bowden GT, Trosko JE, Shapas BG, Boutwell RK. 1975. Excision of pyrimidine dimers from epidermal DNA and nonsemiconservative epidermal DNA synthesis following ultraviolet irradiation of mouse skin. Cancer Res 35:3599-3607.

Breuer B, Kash KM, Rosenthal G, Diemer K, Osborne MP, Miller DG. 1993. Reporting bilaterality status in first-degree relatives with breast cancer: a validity study. Genet Epidemiol 10:245-256.

Butlin HT. 1892. Cancer of the scrotum in chimney-sweeps and others. Lecture I. Secondary cancer without primary cancer. Br Med J 1:1341-1346.

Butlin HT. 1892. Cancer of the scrotum in chimney-sweeps and others. Lecture II. Why foreign sweeps do not suffer from scrotal cancer. Br Med J 2:1-6.

Butlin HT. 1892. Cancer of the scrotum in chimney-sweeps and others. Lecture III. Tar and paraffin cancer. Br Med J 2:66-71.

Cabot S, Shear N, Shear MJ. 1940. Studies in carcinogenesis. XI. Development of skin tumors in mice painted with 3:4-benzpyrene and creosote oil fractions. Am J Pathol 16:301-312.

California Environmental Protection Agency (CalEPA). 1994. Criteria for Carcinogens. Appendix 1, Sections 1 and 2, Update. November 1, 1994.

Cameron G, Naylor M, Morris R, Haynes A, Patrick KE, Lee M, Fischer SM. 1992. Tumor promoter-induced release and metabolism of arachidonic acid: comparison between mouse and human epidermal cells. Toxicol In Vitro 6:109-118.

Carmichael PL, She M, Phillips DH. 1991. DNA adducts in human and mouse skin maintained in short-term culture and treated with petrol and diesel engine lubricating oils. Cancer Lett 57:229-235.

Chapman PH, Rawlins MD, Rogers S, Shuster S. 1977. Aryl hydroxylase activity in psoriatic skin. Br J Clin Pharmacol 4:644.

Chida K, Kuroki T. 1984. Inhibition of DNA synthesis and sugar uptake and lack of induction of ornithine decarboxylase in human epidermal cells treated with mouse skin tumor promoters. Cancer Res 44:875-879.

Chouroulinkov I, Lasne C, Phillips D, Grober P. 1988. Sensitivity of the skin of different mouse strains to the promoting effect of 12-*O*-tetradecanoyl-phorbol-13-acetate. Bull Cancer 75:557-565.

Church R. 1958. The prospect of psoriasis. Br J Dermatol 70:139 (as cited in Durham and Morgan 1974).

Cope FO, Wagner FJ, Conway T, Burns PD, Johnston D, Murphy P, Triggs G, Wille JJ. 1988. Tumorigenic and molecular characterization of novel phorbol ester-resistant and -sensitive lines of mice. Mol Carcinog 1:116-124.

Corsini E, Restani P, Galli C, Marinovich M. 1990. Different effects of TPA on two skin-derived cell lines: murine (HEL-30) and human (NCTC) epidermal cells. Exp Cell Res 191:129-132.

Cox DR, Oakes D. 1984. Analysis of Survival Data. Chapman and Hall, New York. pp. 40-41.

Cunico RL, Maibach HI, Khan H, Bloom E. 1977. Skin barrier properties in the newborn, transepidermal water loss and carbon dioxide emission rates. Biol Neonate 32:177-182.

Dankovic DA, Wright CW, Zangar RC, Springer DL. 1989. Complex mixture effects on the dermal absorption of benzo(a)pyrene and other polycyclic aromatic hydrocarbons from mouse skin. J Appl Toxicol 9:239-244.

Das M, Asokan P, Don P, Krueger GG, Bickers DR, Mukhtar H. 1986a. Carcinogen metabolism in human skin grafted onto athymic nude mice: a model system for the study of human skin carcinogenesis. Biochem Biophys Res Commun 138:33-39.

Das M, Mukhtar H, DelTito B, Marcelo CL, Bickers DR. 1986b. Clotrimazole, an inhibitor of benzo(a)pyrene metabolism and its subsequent glucoronidation, sulfation, and macromolecular binding in BALB/c mouse cultured keratinocytes. J Invest Dermatol 87:4-10.

Day R, Schudiero D, Dimattina M. 1978. Excision repair by human fibroblasts of DNA damaged by *r*-7, *t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene. Mutat Res 50:383-394.

DeLeo VA, Scheide S, Horlich H, Hanson D, Southern W, Harber LC, Weinstein IB. 1986. The effect of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) on phospholipid metabolism of human epidermal keratinocytes in culture. Carcinogenesis 7:937-942.

DiGiovanni J. 1989. Metabolism of polycyclic aromatic hydrocarbons and phorbol esters by mouse skin: relevance to mechanism of action and trans-species/strain carcinogenesis. Prog Clin Biol Res 298:167-199.

Dodd WA. 1993. Tars. Their role in the treatment of psoriasis. Dermatol Clin 11:131-135.

Dotto GP, Gilman MZ, Maruyama M, Weinberg RA. 1986. C-myc and c-fos expression in differentiating mouse primary keratinocytes. EMBO J 5:2853-2857.

Drake LA, Ceilley RI, Cornelison RL, Dobes WA, Dorner W, Goltz RW, Lewis CW, Salasche SJ, Turner MLC. 1993. Guidelines of care for psoriasis. J Am Acad Dermatol 28:632-637.

Durham GA, Morgan JK. 1974. A 7-year follow-up study of ninety patients with psoriasis. Br J Dermatol 91:7-11.

Dybdahl M, Frentz G, Vogel U, Wallin H, Nexo BA. 1999. Low DNA repair is a risk factor in skin carcinogenesis: a study of basal cell carcinoma in psoriasis patients. Mutat Res 433:15-22.

Emmett EA. 1986. Cutaneous and ocular hazards of roofers. Occup Med 1:307-322.

Epstein WL, Maibach HI. 1965. Cell renewal in human epidermis. Arch Dermatol 92:462-468.

Evanoff BA, Gustavsson P, Hogstedt C. 1993. Mortality and incidence of cancer in a cohort of Swedish chimney sweeps: an extended follow up study. Br J Ind Med 50:450-459.

Everall JD, Dowd PM. 1978. Influence of environmental factors excluding ultra violet radiation on the incidence of skin cancer. Bull Cancer 65:241-248.

Finnen MJ, Shuster S, Lawrence CM, Rawlins MD. 1983. Aryl hydrocarbon hydroxylase activity and psoriasis. Biochem Pharmacol 32:1707-1711.

Fisher REW. 1953. Occupational skin cancer in a group of tar workers. Arch Ind Hyg 7:12-18.

Fischer SM, Viaje A, Mills GD, Wong EW, Weeks CE, Slaga TJ. 1984. The growth of cultured human foreskin keratinocytes is not stimulated by a tumor promoter. Carcinogenesis 5:109-112.

Fischer SM, Jasheway DW, Klann RC, Butler AP, Patrick KE, Baldwin JK, Cameron GS. 1989. Correlation of phorbol ester promotion in the resistant C57BL/6J mouse with sustained hyperplasia but not ornithine decarboxylase or protein kinase C. Cancer Res 49:6693-6699.

Fischer SM, Lee ML, Maldve RE, Morris RJ, Trono D, Burow DL, Butler AP, Pavone AM, Warren B. 1993. Association of protein kinase C activation with induction of ornithine decarboxylase in murine but not human keratinocyte cultures. Mol Carcinog 7:228-237.

Fox C, Selkirk J, Price F, et al. 1975. Metabolism of benzo(a)pyrene by human epithelial cells in vitro. Cancer Res 35:3551-3557.

Fraunhofer Institute of Toxicology. 1997. Dermal Carcinogenicity Study of Two Coal Tar Products (CTP) by Chronic Epicutaneous Application in Male CD-1 Mice (78 Weeks). Final Report. Fraunhofer Institute of Toxicology, Federal Republic of Germany. October 1997.

Furstenberger G, Marks F. 1978. Indomethacin inhibition of cell proliferation induced by the phorbol ester TPA is reversed by prostaglandin E2 in mouse epidermis in vivo. Biochem Biophys Res Commun 84:1103-1111.

Furstenberger G, Gross M, Marks F. 1989. Eicosanoids and multistage carcinogenesis in NMRI mouse skin: role of prostaglandins E and F in conversion (first stage of tumor promotion) and promotion (second stage of tumor promotion). Carcinogenesis 10:91-96.

Gibson-D'Ambrosio RE, Leong Y, D'Ambrosio SM. 1983. DNA repair following ultraviolet and n-ethyl-n-nitrosourea treatment of cells cultured from human fetal brain, intestine, kidney, liver, and skin. Cancer Res 43:5846-5850.

Godschalk RWL, Ostertag JU, Moonen EJC, Neumann HAM, Kleinjans JCS, Van Schooten FJ. 1998. Aromatic DNA adducts in human white blood cells and skin after dermal application of coal tar. Cancer Epidemiol Biomarkers Prev 7:767-773.

Goldstein LS, Weyand EH, Safe S, Steinberg M, Culp SJ, Gaylor DW, Beland FA, Rodriguez LV. 1998. Tumors and DNA adducts in mice exposed to benzo(a)pyrene and coal tars: implications for risk assessment. Environ Health Perspect 106(Suppl 6):1325-1330.

Graem N. 1986. Epidermal changes following application of 7,12-dimethylbenz[a]anthracene and 12-*O*-tetradecanoylphorbol-13-acetate to human skin. Cancer Res 46:278-284.

Grimmer G, Brune H, Deutsch-Wenzel R, Dettbarn G, Misfeld J, Abel U, Timm J. 1984. The contribution of polycyclic aromatic hydrocarbons to the carcinogenic impact of emission condensate from coal-fired residential furnaces evaluated by topical application to the skin of mice. Cancer Lett 23:167-176.

Grupper C, Berretti B. 1980. Tar, ultraviolet light, PUVA, and cancer. J Am Acad Dermatol 3:643-646.

Gupta KP, Mehrotra NK. 1990. Assessment of ornithine decarboxylase (ODC) induction potential of cutting oils over mouse skin. Bull Environ Contam Toxicol 44:715-720.

Hall M, Grover P. 1988. Steroselective aspects of the metabolic activation of benzo[a]pyrene by human skin in vitro. Chem Biol Interact 64:281-296.

Halmekyto M, Syrjanen K, Janne J, Alhonen L. 1992. Enhanced papilloma formation in response to skin tumor promotion in transgenic mice overexpressing the human ornithine decarboxylase gene. Biochem Biophys Res Commun 187:493-497.

Halprin KM, Comerford M, Taylor JR. 1982. Cancer in patients with psoriasis. J Am Acad Dermatol 7:633-638.

Hammond EC, Selikoff IJ, Lawther PL, Seidman H. 1976. Inhalation of benzopyrene and cancer in man. Ann NY Acad Sci 271:116-124.

Harris CC. 1987. Human tissues and cells in carcinogenesis research. Cancer Res 47:1-10.

Heller I. 1930. Occupational cancers. J Ind Hyg 12:169-197.

Hemminki K. 1993. DNA adducts, mutations and cancer. Carcinogenesis 14:2007-2012.

Henry SA. 1937. The study of fatal cases of cancer of the scrotum from 1911 to 1935 in relation to occupation, with special reference to chimney sweeping and cotton mule spinning. Am J Cancer 31:28-57.

Henry SA. 1947. Occupational cutaneous cancer attributable to certain chemicals in industry. Br Med Bull 4:389-401 (As cited in IARC 1984).

Hillebrand GG, Winslow MS, Benzinger MJ, Heitmeyer DA, Bissett DL. 1990. Acute and chronic ultraviolet radiation induction of epidermal ornithine decarboxylase activity in hairless mice. Cancer Res 50:1580-1584.

Hoffman FL. 1928. Mule-spinners' cancer. US Monthly Labor Rev 27:27-45.

Hukkelhoven M, Vromans E, Vermorken A, Bloemendal H. 1982. Formation of dihydrodiol metabolites of benzo(a)pyrene in cultured human and murine skin cells. Anticancer Res 2:89-94.

Imamoto A, Wang X-J, Fujiki H, Walker SE, Beltran LM, DiGiovanni J. 1993. Comparison of 12-*O*-tetradecanoylphorbol-13-acetate and telocidin for induction of epidermal hyperplasia, activation of epidermal PKC isozymes and skin tumor promotion in SENCAR and C57BL/6 mice. Carcinogenesis 14:719-724.

International Agency for Research on Cancer (IARC). 1984. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol 34. Polynuclear Aromatic Compounds, Part 3. Industrial Exposures in Aluminum Production, Coal Gasification, Coke Production, and Iron and Steel Founding. IARC, Lyon, France.

International Agency for Research on Cancer (IARC). 1985. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol 35. Polynuclear Aromatic Compounds, Part 4. Bitumens, Coal-tars and Derived Products, Shale-oils and Soots. IARC, Lyon, France.

Iversen O, Bjerknes R, Devik F. 1968. Kinetics of cell renewal, cell migration and cell loss in the hairless mouse dorsal epidermis. Cell Tissue Kinet 1:351-367.

Jacob J, Brune H, Gettbarn G, Grimmer D, Heinrich U, Mohtashampiur E, Norpoth K, Pott F, Wenzel-Hartung R. 1989. Urinary and faecal excretion of pyrene and hydroxypyrene by rats after oral, intraperitoneal, intratracheal or intrapulmonary application. Cancer Lett 46:15-20.

Jansen AP, Colburn NH, Verma AK. 1999. Tumor promoter-induced ornithine decarboxylase gene expression occurs independently of AP-1 activation. Oncogene 18:5806-5813.

Jemec GBE, Østerlind A. 1994. Cancer in patients treated with coal tar: a long-term follow up study. J Eur Acad Dermatol Venereol 3:153-156.

Jones SK, Mackie RM, Hole DJ, Gillis CR. 1985. Further evidence of the safety of tar in the management of psoriasis. Br J Dermatol 113:97-101.

Jongeneelen FJ, Bos RP. 1990. Excretion of pyrene and hydroxypyrene in urine. Letters to the editor. Cancer Lett 51:175-179.

Jongeneelen FJ, Bos RP, Anzion RBM, Theuws JLG, Henderson PT. 1986. Biological monitoring of polycyclic aromatic hydrocarbons. Scand J Work Environ Health 12:137-143.

Kanzler MH, Gorsulowsky DC. 1993. Efficacy of topical 5% liquor carbonis detergens vs. its emollient base in the treatment of psoriasis. Br J Dermatol 129:310-314.

Karlehagen S, Andersen A, Ohlson C-G. 1992. Cancer incidence among creosote-exposed workers. Scand J Work Environ health 18:26-29.

Katiyar SK, Agarwal R, Mukhtar H. 1996. Inhibition of tumor promotion in SENCAR mouse skin by ethanol extract of *Zingiber officinale* Rhizome. Cancer Res 56:1023-1030.

Kennard MD, Kang D-C, Montgomery RL, Butler AP. 1995. Expression of epidermal ornithine decarboxylase and nuclear proto-oncogenes in phorbol ester tumor promotion-sensitive and -resistant mice. Mol Carcinog 12:14-22.

Kinoshita N, Gelboin H. 1972. The role of aryl hydrocarbon hydroxylase in 7,12-dimethylbenz(a)anthracene skin tumorigenesis: on the mechanism of 7,8-benzoflavone inhibition of tumorigenesis. Cancer Res 32:1329-1339.

Klein-Szanto AJP, Conti CJ, Aldaz CM. Skin and oral mucosa. 1991. In: Haschek MW, Rousseauc CG, eds. Handbook of Toxicologic Pathology. Academic Press, Inc., New York. pp. 165-193.

Kouri RE, Ratrie H, Whitmire CE. 1973. Evidence of a genetic relationship between susceptibility to 3-methylcholanthrene-induced subcutaneous tumors and inducibility of aryl hydrocarbon hydroxylase. J Natl Cancer Inst 51:197-200.

Kuroki T, Chida K, Hosomi J, et al. 1989. Use of human epidermal cells in the study of carcinogenesis. J Invest Dermatol 92(5 Suppl):271S-274S.

Lane CG, Crawford CM. 1937. Psoriasis. A statistical investigation of 231 cases. Arch Dermatol Syphilol 35:105 (as cited in Durham and Morgan 1974).

Lawrence CM, Finnen MJ, Shuster S. 1984. Effect of coal tar on cutaneous aryl hydrocarbon hydroxylase induction and anthralin irritancy. Br J Dermatol 110:671-675.

Leadon SA, Sumerel J, Minton TA, Tischler A. 1995. Coal tar residues produce both DNA adducts and oxidative DNA damage in human mammary epithelial cells. Carcinogenesis 6:3021-3026.

Leong LYO. 1990. How I use coal tar in dermatology. Singapore Med J 31:614-615.

Letzel S, Drexler H. 1998. Occupationally related tumors in tar refinery workers. J Am Acad Dermatol 39:712-720.

Levin W, Conney A, Alvares A, et al. 1972. Induction of benzo[a]pyrene hydroxylase in human skin. Science 176:419-420.

Lewis JG, Adams DO. 1987. Early inflammatory changes in the skin of SENCAR and C57BL/6 mice following exposure to 12-*O*-tetradecanoylphorbol-13-acetate. Carcinogenesis 8:889-898.

Lijinsky W, Saffiotti U, Shubik P. 1957. A study of the chemical constitution and carcinogenic action of creosote oil. J Natl Cancer Inst 18:687-692.

Lijinsky W, Domsky I, Mason G, Ramahi HY, Safavi T. 1963. The chromatographic determination of trace amounts of polynuclear hydrocarbons in petrolatum, mineral oil, and coal tar. Anal Chem 35:952-956.

Loprinzi CL, Verma AK. 1984. Effects of local anesthetics on phorbol ester - induced ornithine decarboxylase activity in mouse and human skin. Anticancer Res 4:363-368.

Loprinzi CL, Verma AK. 1985. Dose and schedule of oral retinoic acid and indomethacin needed to effectively inhibit phorbol ester-induced epidermal ornithine decarboxylase activity. Carcinogenesis 6:1589-1592.

Loprinzi CL, Verma AK, Boutwell RK, Carbone PP. 1985. Inhibition of phorbol ester-induced human epidermal ornithine decarboxylase activity by oral compounds: a possible role in human chemoprevention studies. J Clin Oncol 3:751-757.

Lowe NJ. 1981. Ultraviolet light and epidermal polyamines. J Invest Dermatol 77:147-153.

Lowe NJ, Connor MJ, Breeding J, Chalet M. 1982. Inhibition of ultraviolet-B epidermal ornithine decarboxylase induction and skin carcinogenesis in hairless mice by topical indomethacin and triamcinolone acetonide. Cancer Res 42:3941-3943.

Lueke AW. 1907. Epithelioma in carbon workers. Cleveland Med J 6:199-202.

Mahlum D. 1983. Inhibition/promotion studies with coal-derived liquids. J Appl Toxicol 3:31-34.

Maier H, Schemper M, Ortel B, Binder M, Tanew A, Honigsmann H. 1996. Skin tumors in photochemistry for psoriasis: a single-center follow-up of 496 patients. Clin Lab Invest 193:185-191.

Marks F, Furstenberger G, Muller-Decker K. 1999. Metabolic targets of cancer chemoprevention: interruption of tumor development by inhibitors of arachidonic acid metabolism. Recent Results Cancer Res 151:45-67.

Maughan W, Muller S, Perry H, Pittelkow M, O'Brien P. 1980. Incidence of skin cancers in patients with atopic dermatitis treated with coal tar. J Am Acad Dermatol 3:612-615.

Meier JR, Warshawsky D. 1994. Comparison of blood protein and target organ DNA and protein binding following topical application of benzo(a)pyrene and 7*H*-dibenzo(c,g)carbazole to mice. Carcinogenesis 15:2233-2240.

Menter A, Cram DL. 1983. The Goeckerman regimen in two psoriasis day care centers. J Am Acad Dermatol 9:59-65.

Milo GE, Noyes I, Donahoe J, Weisbrode S. 1981. Neoplastic transformation of human epithelial cells in vitro after exposure to chemical carcinogens. Cancer Res 41:5096-5102.

Modly C, Das M, Don P, et al. 1986. Capsaicin as an in vitro inhibitor of benzo(a)pyrene metabolism and its DNA binding in human and murine keratinocytes. Drug Metab Dispos 14:413-416.

Moody RP, Nadeau B, Chu I. 1995. In vivo and in vitro dermal absorption of benzo(a)pyrene in rat, guinea pig, human and tissue-cultured skin. J Dermatol Sci 9:48-58.

Mukhtar H, Das M, Bickers DR. 1986. Skin tumor initiating activity of therapeutic crude coal tar as compared to other polycyclic aromatic hydrocarbons in SENCAR mice. Cancer Lett 31:147-151.

Muller SA, Perry HO. 1984. The Goeckerman treatment in psoriasis: six decades of experience at the Mayo Clinic. Cutis 34:265-269.

Muller-Decker K, Kopp-Schneider A, Marks F, Seibert K, Furstenberger G. 1998. Localization of prostaglandin H synthase isoenzymes in murine epidermal tumors: suppression of skin tumor promotion by inhibition of prostaglandin H synthase-2. Mol Carcinog 23:36-44.

Muller-Decker K, Scholz K, Marks F, Furstenberger G. 1995. Differential expression of prostaglandin H synthase isozymes during multistage carcinogenesis in mouse epidermis. Mol Carcinog 12:31-41.

Nakadate T, Yamamoto S, Ishii M, Kato R. 1982. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced epidermal ornithine decarboxylase activity by phospholipase A_2 inhibitors and lipoxygenase inhibitor. Cancer Res 42:2841-2845.

Nakadate T, Yamamoto S, Aizu E, Kato R. 1989. Inhibition of lipoxygenase inhibitors of 7-bromomethylbenz(a)anthracene-caused epidermal ornithine decarboxylase induction and skin tumor promotion in mice. Carcinogenesis 10:2053-2057.

Nakayama J, Yuspa SH, Poirier MC. 1984. Benzo(a)pyrene-DNA adduct formation and removal in mouse epidermis *in vivo* and *in vitro*: relationship of DNA binding to initiation of skin carcinogenesis. Cancer Res 44:4087-4095.

Nemoto O, Koizumi H, Aoyagi T. 1986. A novel synthetic vitamin-A-like compound (a polyprenoic acid derivative: E-5166) inhibits UVB-stimulated epidermal ornithine decarboxylase activity. Arch Dermatol Res 278:407-409.

Nesnow S, Triplett LL, Slaga TJ. 1982. Comparative tumor-initiating activity of complex mixtures from environmental particulate emissions on SENCAR mouse skin. J Natl Cancer Inst 68:829-834.

Nielsen. 1999. Nielsen Household Panel Distribution Report. Consumer stated usage of Denorex shampoo.

Niggli HJ, Rothlisberger R. 1988. Cyclobutane-type pyrimidine photodimer formation and induction of ornithine decarboxylase in human skin fibroblasts after UV irradiation. J Invest Dermatol 91:579-584.

NIOSH. 1979. Tumors Induced in C3H/HeJ Mice by Coal Tar Neutral Subfractions. Technical Report. U.S. Department of Health Education and Welfare. November 1979.

O'Brien TG. 1976. The induction of ornithine decarboxylase as an early, possibly obligatory, event in mouse skin carcinogenesis. Cancer Res 36:2644-2653.

O'Brien TG, Dzubow L, Dlugosz AA, Gilmour SK, O'Donnell K, Hietala O. 1989. Regulation of ornithine decarboxylase in normal and neoplastic mouse and human epidermis. Prog Clin Biol Res 298:213-231.

Oguro T, Liu J, Klaassen CD, Yoshida T. 1998. Inhibitory effect of oleanolic acid on 12-*O*-tetradecanoylphorbol-13-acetate-induced gene expression in mouse skin. Toxicol Sci 45:88-93.

Ordman AB, Cleaveland JS, Boutwell RK. 1985. 12-*O*-Tetradecanoylphorbol-13-acetate promotes tumors prior to initiation in two-stage promotion. Cancer Lett 29:79-84.

Pannatier A, Jenner P, Testa B, Etter J. 1978. The skin as a drug metabolizing organ. Drug Metab Rev 8:319-343.

Patrick E, Maibach H. 1994. Dermatotoxicology. In: Hayes AW, ed. Principles and Methods of Toxicology, 3rd ed. Raven Press, Ltd., New York. pp. 767-803.

Paulsen JE, Astrup EG. 1983. Effects of single applications of 12-*O*-tetradecanoylphorbol-13-acetate, mezerein, or ethylphenylpropiolate on DNA synthesis and polyamine levels in hairless mouse epidermis. Cancer Res 43:4126-4131.

Pelkonen O, Raunio H. 1983. Genetically determined differences in the response to carcinogens of aryl hydrocarbon hydroxylase and ornithine decarboxylase. IARC Sci Publ 51:157-172.

Perry HO, Soderstrom CW, Schulze RW. 1968. The Goeckerman treatment of psoriasis. Arch Dermatol 98:178-182.

Phillips DH, Schoket B, Hewer A, Grover PL. 1990. DNA adduct formation in human and mouse skin by mixtures of polycyclic aromatic hydrocarbons. IARC Sci Publ 104:223-229.

Pion IA, Koenig KL, Lim HW. 1995. Is dermatologic usage of coal tar carcinogenic? A review of the literature. Dermatol Surg 21:227-231.

Pittelkow M, Perry H, Muller S, Maughan W, O'Brien P. 1981. Skin cancer in patients with psoriasis treated with coal tar. Arch Dermatol 117:465-468.

Pott. 1775. (As cited in Butlin 1892, Lecture I).

Prunieras M, Delescluse C, Bourin M-C. 1982. Hyperplasia and tumor promotion. Int J Dermatol 21:256-258.

Reifenrath WG, Chellquist EM, Shipwash EA, Jederberg WW, Krueger GG. 1984. Percutaneous penetration in the hairless dog, weanling pig, and grafted anthymic nude mouse: evaluation of models for predicting skin penetration man. Br J Dermatol 111:123-135.

Reiners JJ, Nesnow S, Slaga TJ. 1984. Murine susceptibility to two-stage skin carcinogenesis is influenced by the agent used for promotion. Carcinogenesis 5:301-307.

Rhim JS, Fujita J, Arnstein P, Aaronson SA. 1986. Neoplastic conversion of human keratinocytes by adenovirus 12-SV40 virus and chemical carcinogens. Science 232:385-387.

Rice RH, Cohen DE. 1996. Toxic responses of the skin. In: Casarett and Doull's Toxicology. The Basic Science of Poisons. 5th ed. McGraw-Hill, New York. pp. 529-546.

Rønneberg A, Andersen A. 1995. Mortality and cancer morbidity in workers from an aluminum smelter with prebaked carbon anodes–part II: cancer morbidity. Occup Environ Med 52:250-254.

Ross P. 1948. Occupational skin lesions due to pitch and tar. Br Med J 2:369-374.

Roy TA, Krueger AJ, Taylor BB, Mauro DM, Goldstein LS. 1998. Studies estimating the dermal bioavailability of polynuclear aromatic hydrocarbons from manufactured gas plant tar-contaminated soils. Environ Sci Technol 32:3113-3117.

Roy TA, Yang JJ, Czerwinski MH. 1987. Evaluating the percutaneous absorption of polynuclear aromatics using *in vivo* and *in vitro* techniques and structure activity relationships. In: Goldberg AM, Principal ML, eds. Alternative Methods in Toxicology. Vol . 5. In Vitro Toxicology: Approaches to Validation. Mary Ann Liebert, New York. pp. 471-482.

Rozman KK, Klaassen CD. 1996. Absorption, distribution, and excretion of toxicants. In: Casarett and Doull's Toxicology. The Basic Science of Poisons. 5th ed. McGraw-Hill, New York. pp. 91-112.

Ruhl KK, Pomodor MM, Rhim JS, Tuan RS, Hickok NJ. 1994. Post-transcriptional suppression of human ornithine decarboxylase gene expression by phorbol esters in human keratinocytes. J Invest Dermatol 103:687-692.

Sala M, Lasne C, Lu Y, Chouroulinkov I. 1987. Morphological transformation in three mammalian cell systems following treatment with 6-nitrochrysene and 6-nitrobenzoapyrene. Carcinogenesis 8:503-507.

Santella RM, Nunes MG, Blaskovic R, Perera FP, Tang D, Beachman A, Lin H-J, DeLeo VA. 1994. Quantitation of polycyclic aromatic hydrocarbons, 1-hydroxypyrene, and mutagenicity in urine of coal tar-treated psoriasis patients and untreated volunteers. Cancer Epidemiol Biomarkers Prev 3:137-140.

Sato S, Tomita I. 1998. Response differences among mouse strains in DNA damage and skin carcinogenicity of 7,12-dimethylbenz(a)anthracene are due to inducible aryl hydrocarbon hydroxylase activity. Biol Pharm Bull 21:90-92.

Schaefer H, Zesch A, Stuttgen G. 1977. Penetration, permeation, and absorption of triamcinolone acetonide in normal and psoriatic skin. Arch Dermatol Res 258:241-249.

Schamberg JF. 1910. Cancer in tar workers. J Cutan Dis 28:644 (As cited in Heller 1930).

Schoket B, Hewer A, Grover PL, Phillips DH. 1988a. Covalent binding of components of coal-tar, creosote and bitumen to the DNA of the skin and lung of mice following topical application. Carcinogenesis 9:1253-1258.

Schoket B, Hewer A, Grover PL, Phillips DH. 1988b. Formation of DNA adducts in human skin maintained in short-term organ culture and treated with coal-tar, creosote or bitumen. Int J Cancer 42:622-626.

Schoket B, Horkay I, Kosa A, Paldeak L, Hewer A, Grover PL, Phillips DH. 1990. Formation of DNA adducts in the skin of psoriasis patients, in human skin and lung following topical application of coal-tar and juniper tar. J Invest Dermatol 94:241-246.

Scotto J, Kopf AW, Urbach F. 1974. Non-melanoma skin cancer among Caucasians in four areas of the United States. Cancer 34:1333-1338.

Scotto J, Fears T, Lisiecki E, Radosevich S. 1980. Incidence of Nonmelanoma Skin Cancer in the United States, 1977-78. DHEW Publication No. 80-2154. U.S. Department of Health, Education, and Welfare, National Cancer Institute.

Scotto J, Fears TR, Fraument JF. 1983. Incidence of Nonmelanoma Skin Cancer in the United States. NIH Publication No. 82-2433. U.S. Department of Health and Human Services, Bethesda MD.

Sexton RJ. 1960. The hazards to health in the hydrogenation of coal. Arch Environ Health 1:208-231.

Shabad LM, Khesina AJ, Linnik AB, Serkovskaya GS. 1970. Possible carcinogenic hazards of several tars and locacorten-tar ointment (spectro-fluorescent investigations and experiments in animals). Int J Cancer 6:314-318.

Sherson D, Svane O, Lynge E. 1991. Cancer incidence among foundry workers in Denmark. Arch Environ Health 46:75-81.

Silverman A, Menter A, Hairston JL. 1995. Tars and anthralins. Dermatol Clin 13:817-833.

Singer EJ, Wegmann PC, Lahman MD, Christensen MS, Vinson LJ. 1971. Barrier development, ultrastructure, and sulfhydryl content of the fetal epidermis. J Soc Cosmet Chem 22:119-137.

Singh R, Tucek M, Maxa K, Tenglerova J, Weyand EH. 1995. A rapid and simple method for the analysis of 1-hydroxypyrene glucuronide: a potential biomarker for polycyclic aromatic hydrocarbon exposure. Carcinogenesis 16:2909-2915.

Slaga TJ. 1986. SENCAR mouse skin tumorigenesis model versus other strains and stocks of mice. Environ Health Perspect 68:27-32.

Slaper H, van der Leun JC. 1987. Human exposure to ultraviolet radiation: quantitative modelling of skin cancer incidence. In: Passchier WF, Bosnjakovic BFM, eds. Human Exposure to Ultraviolet Radiation: Risks and Regulations. Elsevier Science Publishers, New York. pp. 5-20.

Soballe PW, Montone KT, Satyamoorthy K, Nesbit M, Harlyn M. 1996. Carcinogenesis in human skin grafted to SCID mice. Cancer Res 56:757-764.

Soriani M, Luscher P, Tyrrell R. 1999. Direct and indirect modulation of ornithine decarboxylase and cyclooxygenase by UVB radiation in human skin cells. Carcinogenesis 20:727-732.

Spinelli JJ, Band PR, Svirchev LM, Gallagher RP. 1991. Mortality and cancer incidence in aluminum reduction plant workers. J Occup Med 33:1150-1155.

Stern RS. 1989. Risk assessment of PUVA and cyclosporine. Arch Dermatol 125:545-547.

Stern RS. 1995. Epidemiology of psoriasis. Dermatol Clin 13:717-722.

Stern RS, Laird N. 1994. The carcinogenic risk of treatments for severe psoriasis. Cancer 73:2759-2764.

Stern RS, Zierler S, Parrish JA. 1980. Skin carcinoma in patients with psoriasis treated with topical tar and artificial ultraviolet radiation. Lancet 1:732-735.

Stern RS, Laird N, Melski J, Parrish JA, Fitzpatrick TB, Bleich HL. 1984. Cutaneous squamous-cell carcinoma in patients treated with PUVA. N Engl J Med 310:1156-1161.

Stern RS, Scotto J, Fears TR. 1985. Psoriasis and susceptibility to nonmelanoma skin cancer. J Am Acad Dermatol 12:67-73.

Stern RS, Liebman E, Vakeva L, PUVA Follow-up Study. 1998. Oral psoralen and ultraviolet-A light (PUVA) treatment of psoriasis and persistent risk of nonmelanoma skin cancer. J Natl Cancer Inst 90:1278-1284.

Storm J, Collier S, Stewart R, Bronaugh R. 1990. Metabolism of xenobiotics during percutaneous penetration: Role of absorption rate and cutaneous enzyme activity. Fundam Appl Toxicol 15:132-141.

Suda M, Tanaka K, Sakuma Y, Yasoda A, Ozasa A, Fukata J, Tanaka I, Narumiya S, Nakao K. 2000. Prostaglandins E_2 (PGE_2) induces the c-fos and c-jun expressions via the EP_1 subtype of PGE receptor in mouse osteoblastic MC3T3-E1 cells. Calcif Tissue Int 66:217-223.

Swanger D, Mahlum DD, Springer DL. 1983. Ornithine decarboxylase induction by chemically complex liquids from two solvent refined coal processes. Carcinogenesis 4:805-810.

Taichman LB, Setlow RB. 1979. Repair of ultraviolet light damage to the DNA of cultured human epidermal keratinocytes and fibroblasts. J Invest Dermatol 73:217-219.

Takigawa M, Verma AK, Simsiman RC, Boutwell RK. 1983. Inhibition of mouse skin tumor promotion and of promoter-stimulated epidermal polyamine biosynthesis by á-difluoromethylornithine. Cancer Res 43:3732-3738.

Thompson S, Slaga TJ. 1976. Mouse epidermal aryl hydrocarbon hydroxylase. J Invest Dermatol 66:108-111.

Toppmeyer Research, Inc. 1994. Denorex Usage Study. Job #02-01-94. Toppmeyer Research, Inc., Great Neck, NY.

Urano K, Katakai Y, Tokuda Y, Ueyama Y, Nomura T, Yamamoto S. 1995. Failure of genotoxic carcinogens to produce tumors in human skin xenografts transplanted to SCID mice. Carcinogenesis 16:2223-2226.

USDHHS. 1989. Vital Statistics of the United States. Volume II - Mortality, Part A. U.S. Department of Health and Human Services, Hyattsville, MD.

USEPA. 1992. Guidelines for exposure assessment; notice. Fed Regist 57(104):22888-22938.

USEPA. 1996. Proposed Guidelines for Carcinogen Risk Assessment. EPA/600/P-92/003C. Office of Research and Development, Washington, DC. April, 1996.

USEPA. 1997. An Approach for Calculating Risk from Dermal Exposure to Benzo(a)pyrene Based on Point of Contact Effects. Draft.

USEPA. 1999. Science Advisory Board. Review of the USEPA 1996 Proposed Guidelines for Carcinogen Risk Assessment.

USEPA. 2000. Personal communication with Dr. James Cogliano. April 2000.

Van Houten B, Masker WE, Carrier WL, Regan JD. 1986. Quantitation of carcinogen-induced DNA damage and repair in human cells with the UVR ABC excision nuclease from *Escherichia coli*. Carcinogenesis 7:83-87.

Van Rooij J. 1995. An Assessment of the Health Risks in Connection With Using Shampoo Which Contains Tar. Industox Report No. IT-9422.

Van Rooij J, Bodielier-Bade M, Jongeneelen F. 1993a. Estimation of individual dermal and respiratory uptake of polycyclic aromatic hydrocarbons in 12 coke oven workers. Br J Ind Med 50:623-632.

Van Rooij J, DeRoos JHC, Bodelier-Bade MM, Jongeneelen FJ. 1993b. Absorption of polycyclic aromatic hydrocarbons through human skin: differences between anatomical sites and individuals. J Toxicol Environ Health 38:355-368.

Van Rooij J, Vinke E, DeLange J, Bruijnzeel PLB, Bodelier-Bade MM, Noordhoek J, Jongeneelen FJ. 1995. Dermal absorption of polycyclic aromatic hydrocarbons in the blood-perfused pig ear. J Appl Toxicol 15:193-200.

Van Schooten F-J, Godschalk R. 1996. Coal tar therapy. Is it carcinogenic? Drug Safety 15:374-377.

Van Schooten F-J, Moonen EJC, Rheinsburger E, van Agen B, Thijssen HHW, Kleinjans JCS. 1994. Dermal uptake of polycyclic aromatic hydrocarbons after hairwash with coal-tar. Lancet 344:1505-1506.

Verma AK, Rice HM, Boutwell RK. 1977. Prostaglandins and skin tumor promotion: inhibition of tumor promoter-induced ornithine decarboxylase activity in epidermis by inhibitors of prostaglandin synthesis. Biochem Biophys Res Commun 79:1160-1166.

Verma AK, Conrad EA, Boutwell RK. 1980a. Induction of mouse epidermal ornithine decarboxylase activity and skin tumors by 7,12-dimethylbenz(a)anthracene: modulation by retinoic acid and 7,8-benzoflavone. Carcinogenesis 1:607-611.

Verma AK, Ashendel CL, Boutwell RK. 1980b. Inhibition by prostaglandin synthesis inhibitors of the induction of epidermal ornithine decarboxylase activity, the accumulation of prostaglandins, and tumor promotion caused by 12-*O*-tetradecanoylphorbol-13-acetate. Cancer Res 40:308-315.

Verma AK, Hsieh JT, Pong RC. 1988. Mechanisms involved in ornithine decarboxylase induction by 12-*O*-tetradecanoylphorbol-13-acetate, a potent mouse skin tumor promoter and an activator of protein kinase C. Adv Exp Med Biol 250:273-290.

Verma AK, Loprinzi CL, Boutwell RK, Carbone PP. 1985. In vitro induction of human skin ornithine decarboxylase by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate. J Natl Cancer Inst 75:85-90.

Verma AK, Pong R-C, Erickson D. 1986. Involvement of protein kinase C activation in ornithine decarboxylase gene expression in primary culture of newborn mouse epidermal cells and in skin tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate. Cancer Res 46:6149-6155.

Viau C, Carrier G, Vyskocil A, Dodd C. 1995. Urinary excretion kinetics of 1-hydroxypyrene in volunteers exposed to pyrene by the oral and dermal route. Sci Total Environ 163:179-186.

Volkmann. 1875. On tar, paraffin, and soot cancer (chimney-sweeps' cancer). Beitrage zur Chirugie (As cited in Butlin 1892, Lecture III).

Waldron HA, Waterhouse HA, Tessema N. 1984. Scrotal cancer in the West Midlands 1936-76. Br J Ind Med 41:437-444.

Wang JC, Patel BG, Ehmann CW, Lowe N. 1987. The release and percutaneous permeation of anthralin products, using clinically involved and uninvolved psoriatic skin. J Am Acad Dermatol 16:812-821.

Warshawsky D, Barkley W, Bingham E. 1993. Factors affecting carcinogenic potential of mixtures. Fundam Appl Toxicol 20:376-382.

Watanabe M, Watanabe K, Konno K, Sato H. 1975. Genetic differences in the induction of aryl hydrocarbon hydroxylase and benzo(a)pyrene carcinogenesis in C3H/He and DBA/2 strains of mice. Gann 66:217-226.

Watson WP, Smith RJ, Huckle KR, Wright AS. 1988. Human organ culture techniques for the detection and evaluation of genotoxic agents. IARC Sci Publ 89:384-388.

Wester RC, Maibach HI, Bucks DAW, Sedik L, Melendres J, Liao C, DiZio S. 1990. Percutaneous absorption of [^{14}C]DDT and [^{14}C]benzo(a)pyrene from soil. Fundam Appl Toxicol 15:510-516.

Wester RC, Bucks DA, Maibach HI. 1983. In vivo percutaneous absorption of hydrocortisone in psoriatic patients and normal volunteers. J Am Acad Dermatol 8:645-647.

Wester RC, Noonan PK, Cole MP, Maibach HI. 1977. Percutaneous absorption of testosterone in the newborn Rhesus monkey: comparison to the adult. Pediat Res 11:737-739.

Weston A, Hodgson RM, Hewer AJ, Kuroda R, Grover PL. 1985. Comparative studies of the metabolic activation of chrysene in rodent and human skin. Chem-Biol Interact 54:223-242.

Wheldrake J, Marshall J, Ramil J, et al. 1982. Skin carcinogenesis and promoter binding characteristics in different mouse strains. Carcinogenesis 8:805-807.

Wood HB. 1929. Paraffin not productive of cancer. J Cancer Res 13:97 (As cited in Heller 1930).

Wright CW, Later DW, Pelroy RA, Mahlum DD, Wilson BW. 1985. Comparative chemical and biological analysis of coal tar-based therapeutic agents to other coal-derived materials. J Appl Toxicol 5:80-88.

Yamada S, Hirota K, Chida K, Kuorki T. 1988. Inhibition of phorbol ester-caused induction of ornithine decarboxylase and tumor promotion in mouse skin by staurosporine, a potent inhibitor of protein kinase C. Biochem Biophys Res Commun 157:9-15.

Yamamoto S, Kiyoto I, Aizu E, Sasakawa N, Nakaki T, Kato R. 1988. Comparison of some biochemical properties of epidermis in tumor promotion-susceptible and -resistant strains of mice. Jpn J Pharmacol 47:45-49.

Yamamoto S, Aizu E, Jiang H, Nakadate T, Kiyoto I, Wang JC, Kato R. 1991. The potent anti-tumor-promoting agent isoliquiritigenin. Carcinogenesis 12:317-323.

Yamamoto S, Jiang H, Otsuka C, Kato R. 1992. Involvement of prostaglandin E2 in ornithine decarboxylase induction by a tumor-promoting agent, 7-bromomethylbenz(a)anthracene, in mouse epidermis. Carcinogenesis 13:905-906.

Yohn J, Lehman TA, Kurian P, Ribovich M, Milo GE. 1988. Benzo(a)pyrene diol epoxide I modification of DNA in human skin xenografts. J Invest Dermatol 91:363-368.

Young MR, Li JJ, Rincon M, Flavell RA, Sathyanarayana BK, Hunziker R, Colburn N. 1999. Transgenic mice demonstrate AP-1 (activator protein-1) transactivation is required for tumor promotion. Proc Natl Acad Sci 96:9827-9832.

Yuspa SH. 1994. The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis. Cancer Res 54:1178-1189.

APPENDIX A

Summary of Occupational Studies

A.1 Introduction

The body of evidence that suggests occupational exposures to constituents in coal tar and other PAH mixtures results in an increase in skin cancer incidence is predominantly from early anecdotal and case study reports (IARC 1984). In 1775, Percival Pott reported cases of scrotal cancer in individuals employed as chimney sweeps in England (Pott 1775). Following this report, cases of scrotal cancer and skin cancer at other sites, including the head, neck, arms, and hands, were reported in workers employed in other industries including workers in coke ovens, tar distillation, tar and paraffin manufacturing, roofing, creosoting, coal gasification, the cotton industry (mule spinners), and aluminum and steel smelting (summarized in Table A-1). As described in the following sections, the majority of patients presenting with skin cancer had two features in common: 1) employment in an occupation where the potential for exposure to PAHs was high, and 2) some form of chronic skin irritation, either associated with exposure to high concentrations of PAHs or with other sources of irritation such as friction from clothing or phototoxicity from repeated exposures to ultraviolet (UV) sunlight.

A.1.1 Exposure to Soot

Workers employed as chimney sweeps were exposed to PAHs in soot, and early case reports have described cases of scrotal cancer in chimney sweeps employed in England. In these individuals, the occurrence of scrotal cancer was generally coupled with factors in addition to exposure to soot, such as poor hygiene and cell injury resulting from chronic irritation. According to Butlin (1892), scrotal cancer in chimney sweeps was associated with irritation, inflamation, dermatitis and folliculitis of the scrotum and surrounding groin area, followed by the formation of “soot” warts with keratotic horns on the scrotum, some of which upon further irritation, progressed to ulcerated epithelioma. Butlin (1892) also noted that the soot warts did not always progress to malignancy, but rather in the majority of cases regressed, and secondly, epithelioma and warts were never found on the surrounding groin area or on other exposed areas, such as the face or hands, but only on the scrotum.

In contrast, scrotal cancer was rarely, if ever, seen in chimney sweeps employed in other countries, such as Belgium, Germany, and France (Butlin 1892). The reasons cited for the lack

of scrotal cancer in countries other than England were that the clothing worn by chimney sweeps in other countries was more protective and that chimney sweeps in these countries practiced good hygiene. In comparing the attire, cleanliness, and living quarters between the English sweeps and those employed in Belgium, Germany, and France, Butlin (1892) reported that the English sweeps wore clothes made of a thick material but the clothes were dirty and at times torn and tattered. The English sweeps took baths at most once per week, and the living quarters were extremely dirty with a high presence of soot. Sweeps from other countries also wore clothing made of a thick material; however, they usually cleaned the clothes once or twice per week or had more than one set of work attire. Moreover, many of the sweeps in Belgium and Germany wore hoods accompanied by a face wrap instead of a normal cap. Further, sweeps in these countries practiced better hygiene, with some taking two baths per day, one in the shop after work, and another at home. And lastly, the living quarters of the sweeps in countries other than England were clean and free of soot. Consequently, the extent and duration of soot exposure were clearly different.

The most recent epidemiology study conducted for chimney sweeps evaluated the incidence of cancer in a cohort of 5,242 chimney sweeps employed between 1957 and 1987 through the national trade union in Sweden (Evanoff et al. 1993). The average years of employment were 12 ± 12.2 years with a median employment of 9.0 years. No increase in the incidence of skin cancer was found (observed = 4; expected 6.5; SIR = 0.61). As an explanation for the lack of skin cancer in this cohort, the authors of the study mentioned that Swedish sweeps have long had the right to bathe on paid time following work. Thus, hygiene practices were likely better in this cohort than in the English cohort.

A.1.2 Exposure to Coal Tar, Creosote and Pitch

In a number of occupations workers were potentially exposed to coal tar, coal tar fumes, creosote, or pitch dust or fumes including workers at facilities such as gas-works, coal tar distilleries, coke ovens, creosote plants, as well as roofers. The earliest reports of the occurrence of skin cancer in coal tar workers consisted of individual case reports describing cases of scrotal cancer (Butlin 1892). As presented by Butlin (1892), Volkmann (1875) and Bell (1876) reported

three cases and two cases, respectively, of scrotal cancer in workers employed within the tar and paraffin industry. Three cases of scrotal cancer were reported in carbon workers who were potentially exposed to coal tar and pitch (Lueke 1907). Hoffman (1928) reported seven deaths from scrotal cancer out of 1,150 patent fuel workers employed between 1911 and 1920 in England and Wales; and 11 deaths from scrotal cancer out of 54,447 coal gasification workers employed during the same period based on death certificates and census data for 1911. However, Hoffman (1928) noted that scrotal cancer was predominately reported in individuals employed in industries where the potential for irritation was high and that irritation played a role in the formation of scrotal cancer. Thus, as with the chimney sweeps discussed in the previous section, irritation was a factor in the formation of scrotal tumors.

In a more recent study, conducted by Waldron et al. (1984), the incidence of scrotal cancer reported in the West Midland Regional Cancer Registry between 1936 and 1976 was evaluated according to occupation. A total of 344 cases of scrotal cancer were registered, of which only 27 (7.9%) occurred in individuals exposed to coal tar or pitch. Fifty-seven cases (16.6%) of scrotal cancer were reported in individuals with no known exposure to PAHs.

Cases of skin cancer have been reported at sites other than the scrotum in workers in the coal tar industry, with the early evidence based on case reports of workers employed in coal tar distillation facilities. Ball (1885) reported two cases of skin cancer in workers employed in the distillation of tar. Ross (1948) noted that 358 cases of skin cancer in individuals employed in coal tar distillation facilities were reported to the Chief Inspector of Factories in Britain between 1921 and 1945, and 91 cases of skin cancer were reported in individuals employed at gas-works services for the same time period. Of 3,753 total cases of skin cancer reported to the Factory Inspectorate in the UK, Henry (1947) attributed 2,229 cases to coal tar or pitch exposure.

However, there are several points that should be considered when evaluating these reports on the incidence of skin cancer in workers exposed to coal tar and pitch. First, no attempts were made to categorize skin cancer incidence by job position, or, in some cases, by occupation. Secondly, no characterization of exposure or exposure concentrations were provided in the studies that investigated the occurrence of skin cancer in these workers. Because much of the data reported in these studies was based on exposures that occurred before the implementation of

modern industrial hygiene practices, the exposure concentrations for these cohorts may have been very high. It is worth noting that the two studies used by IARC (1984) to illustrate the occurrence of skin cancer in both coal gasification and coke processing presented data extending back to the mid-1800s. However, the exposure data for these occupations reported by IARC (1984) were taken from studies conducted between 1950 and 1980 that measured air concentration. Lastly, most of these occupations included co-exposure to UV-B irradiation, with some workers performing their duties primarily in an outdoor setting where UV-B exposure would be high; and the studies that investigated the occurrence of skin cancer and occupational exposures to coal tar and pitch did not take into account the contribution of UV-B irradiation or any interactions between PAHs and UV-B rays and the observed skin cancer incidence.

In contrast to the early reports from Britain, Heller (1930) suggested that skin cancer resulting from exposure to coke oven tar or coal tar at tar distilleries in the U.S. was low. Heller (1930) evaluated the records of cancer clinics and hospitals and interviewed plant physicians and local physicians treating skin ailments for workers employed at various coal tar distilleries, coke ovens, and coal gasification facilities in the U.S. Of 300 workers employed at ten distillation plants operated by the American Tar Product Company, no cases of skin cancer were reported, although complaints of burns resulting from splashing were typical. Similarly, distillation plants in Chicago primarily producing pitch also reported no cases of skin cancer. Further, in Chicago, the gas company operated eight plants that produced coke oven gas tar, and the physicians serving these plants reported that no cases of skin cancer had been observed. Likewise, no skin cancer cases were reported from the physicians serving the Chicago By-Products Company, which manufactured coke oven tar and by-products, or from the physicians serving many other coke oven and water gas facilities in New York, Boston, Pittsburgh, Chicago and Milwaukee.

According to Heller (1930) the lack of skin cancer observed in workers at the above coal tar distilleries and coke ovens was consistent with the findings reported by Wood (1929). Of 88 coal tar handlers employed at various gas-works or coke oven facilities in Pennsylvania, a few wart-like conditions were found, but no cancer (Wood 1929). Further, the Philadelphia Gas Company also reported no cases of skin cancer out of 750 workers, and no cases of skin cancer were reported in 500 employees at the Barrett distillation plant (Wood 1929).

There were, however, early reports that describe cases of skin cancer in individuals employed in coal tar industries in the U.S. Of 19 cases of skin cancer in individuals exposed to coal tar or pitch treated at Memorial Hospital, New York, and at the New York Skin and Cancer Hospital between 1920 and 1928, four cases were reported for roofers, two cases were reported for employees at a coal tar distillery, one case was reported in a coke oven worker, and 12 cases were reported in gas-works employees (Heller 1930). Likewise, a Cleveland industrial plant that used large amounts of pitch (dust) in the production of carbon and dry batteries reported a total of 21 cases of skin cancer; three scrotal tumors, seven located on the face or lip, and 11 located on the hand, wrists, or arm (Heller 1930). According to Heller (1930), Schamberg (1910) reported five cases of skin cancer in workers exposed to coal tar used to manufacture roofing paper for 13-55 years. Of these five cases, one case was scrotal cancer with preliminary skin irritation, and four cases had skin cancers located on the hands or wrists.

As with the reports from England, for the tumors on sun-exposed areas, exposure to UV-B irradiation was not taken into account in the studies of cohorts in the U.S. Further, data regarding exposures to coal tar were not presented. However, attempts were made to classify the incidence of skin cancer by occupation and job position, particularly in the report concerning the skin cancer incidence at the carbon plant in Cleveland (Heller 1930).

More recent epidemiological studies have evaluated the skin cancer incidence in cohorts of workers in the U.S. as well as other countries who were employed in industries with the potential for exposure to coal tar, pitch and pitch volatiles using skin cancer incidence rates in control populations for comparison. In a study by Sexton (1960), the incidence of skin cancer in a group of 359 workers at a coal hydrogenation plant in West Virginia was evaluated. The data in this report were taken from the required periodic skin inspection reports at the plant. Both precancerous and cancerous lesions were reported. A total of 54 suspected precancerous lesions in 45 workers was identified in this cohort. Of these 54 lesions, 42 were confirmed precancerous. The type and number of lesions reported included pitch acne (3), calcifying epithelioma (1), keratoacanthoma (1), chondrodermatitis (3), keratosis (25), and acanthosis or hyperkeratosis (9). The latency period between first exposure (employment) and the occurrence of the lesions was reported to be between 3.5 and 116 months.

A total of five cases of skin cancer were reported, including four cases of squamous cell carcinoma (SCC) and one case of basal cell carcinoma (BCC). The latency period between employment and the occurrence of the tumors was reported to be between 9 and 116 months. The incidence of skin cancer in this cohort was reported to be significantly increased ($p = 0.032$) when compared to a background incidence rate of 30/100,000 as reported by Dorn and Cutler (Sexton 1960). However, it is important to note that four of the five cases were located on the head, neck or hand, which are sites commonly associated with tumors caused by UV light (Slaper and van der Leun 1987).

A recent study conducted in Germany by Letzel and Drexler (1998) evaluated the incidence of skin cancer in workers who were exposed to coal tar, pitch and distillate products. Briefly, 606 workers (601 men and 5 women) from a tar refinery in Germany who were recognized as having occupationally-induced disease between 1946 and 1996 were evaluated. In 1996, 277 subjects of the original cohort were alive, with a median age of 66 years. The median duration of employment was reported to be 32 years and more than 77% of the workers had been employed for more than 20 years. Estimates of exposure were not provided. A total of 4,754 skin lesions was identified for the persons in the cohort and 4,280 were diagnosed histologically. The majority of lesions were considered precancerous and the authors noted that tar or pitch keratosis was an obligatory precursor step. There were 380 SCCs in 151 individuals and 218 BCCs in 98 individuals identified in the study. Although the authors claim that the role of UV light in this cohort is questionable, more than 90% of the SCCs and BCCs were located in sun-exposed areas. Thus, there is a high probability that UV light did contribute to the formation of skin tumors in this cohort. Letzel and Drexler (1998) note that because of methodical reasons, the exact quantification of the incidence of skin tumors in this cohort was not possible. More specifically, there was no control group suitable for comparison because of the year of birth range (1882-1960). Also, a dermatological monitoring program was initiated in the 1950s. Thus, selection and intervention bias were present and assumed to influence the results, and in the absence of exposure data, this study is inadequate for use in risk assessment.

Sherson et al. (1991) evaluated the cancer incidence in a cohort of 6,144 male foundry workers who had been invited to participate in x-ray examinations in Denmark as part of a

national silicosis survey. There were two x-ray sessions, one between 1967 and 1969 and one between 1972 and 1974. Most of the individuals had been issued personal identification numbers that were linked to the Central Population Register. The few individuals who did not have personal identification numbers were manually traced. The materials to which that the workers were exposed were not reported; however, coke ovens are typically located at foundries, so these workers may have been exposed to high-temperature coke oven tar. Cancer incidence rates were assessed by linking the personal identification numbers with the Denmark Cancer Registry for years 1943-1977. The cancer incidence rates were compared to expected values for the Danish population that were calculated based on five-year age, group, sex, and calender periods (1963-1967, 1968-1972, 1973-1977, 1978-1982). The standard morbidity ratio (SMR) for nonmelanoma skin cancer was 0.93 (65 cases observed/69.86 cases expected), and thus, the authors reported that there was no significant increase in the incidence of skin cancer in this cohort of foundry workers, when compared to the general population.

Recent studies evaluating the incidence of skin cancer in aluminum workers suggest that the risk of skin cancer resulting from exposure to coal tar pitch volatiles (CTPV) is very low. Spinelli et al. (1991) reported on the incidence of nonmelanoma skin cancer in 4,213 aluminum workers employed in British Columbia between 1954 and 1985. Ninety-two percent of the cohort was successfully traced. The expected skin cancer incidence was obtained from the British Columbia Cancer Registry. The Standardized Incidence Ratio (SIR) for nonmelanoma skin cancer was only 0.51 (38 cases observed/74.31 cases expected = 0.51). Thus, the risk of nonmelanoma skin cancer in this cohort of aluminum workers was half that expected for the general population.

A second study reported on the mortality resulting from nonmelanoma skin cancer in workers employed at an aluminum smelter between 1922 and 1975 (Rønneberg and Andersen 1995). Comparisons were made to the expected incidence in the general population. The SIR (observed/expected) for skin cancer in workers employed for less than 3 years was 3.09. However, for workers employed for 3 or more years, the SIR for skin cancer was only 0.79. Thus, there was no increased risk of skin cancer associated with long-term employment at the aluminum smelter.

Skin cancer was evaluated in a cohort of roofers exposed to coal tars and pitches, and bitumens (Hammond et al. 1976). In this study, the number of deaths due to skin cancer in 5,939 roofers and waterproofers from all parts of the U.S. who were members of the United Slate, Tile and Composition Roofers, Damp and Waterproof Workers Association was evaluated. Exposure data were provided as $\mu g/m^3$ and were reported to be from 14 $\mu g/m^3$ in the roof-tarring area to 6,000 $\mu g/m^3$ around the coal tar roofing kettle area. However, the positions for the individuals who died with skin cancer were not identified. Five deaths resulting from skin cancer were observed as opposed to 1.18 expected deaths for each cause in the U.S. male population in comparable calender years. The SMR for skin cancer in this cohort was reported to be greater than 4.0 with a ratio of 1.0 being normal. Thus, the authors concluded that the number of deaths resulting from skin cancer in this cohort was significantly above the expected value for the U.S. male population. However, the study authors did not map the skin tumors to anatomical region and the potential contribution of UV light was not considered.

A.1.3 Exposure to Mineral and Cutting Oils

In addition to chimney sweeps and coal tar workers, scrotal cancer has been observed in workers exposed to mineral oils in the processing of cotton (cotton mule spinners). As presented by Hoffman (1928), of 74,625 men employed as cotton mule spinners between 1911 and 1920 in England and Wales, 100 developed scrotal cancer. Hoffman (1928) concluded that the incidence of scrotal cancer in cotton mule spinners was due to a combination of exposure to PAHs present in the lubricating oils and chronic chemical and mechanical irritation from the trousers. The importance of the mechanical irritation was highlighted by the fact that although wool mule spinners were exposed to PAHs to the same extent as cotton mule spinners, no reports of scrotal cancer had been reported in wool mule spinners. Hoffman (1928) noted that the explanation for the differential incidence rate was that cotton mule spinners operated in a hot, moist environment that predisposed the employee to irritation from the trousers, whereas the wool mule spinners operated in a cool, dry environment. Hence, in addition to the potential contribution of exposure to PAHs, as with the British chimney sweeps, irritation may have been a factor.

In a study conducted by Waldron et al. (1984), the incidence of scrotal cancer reported in the West Midland Regional Cancer Registry between 1936 and 1976 was evaluated according to occupation. A total of 344 cases of scrotal cancer was registered, of which 61% occurred in individuals exposed to mineral oils. The incidence of scrotal cancer in individuals with no known exposure to PAHs was 16.6%. These data qualitatively illustrate the occurrence of scrotal cancer in individuals exposed to mineral oil and cutting oil, as well as in individuals who were not. No quantitative interpretation could be made because the number of individuals in each group (exposed vs. nonexposed) was not reported. Although these data do suggest that an increased incidence of scrotal cancer was associated with exposure to lubricating oils, the authors noted that poor hygiene was likely a contributing factor in the formation of the scrotal tumors.

A.1.4 Exposures to Creosote

The potential skin cancer risk in a cohort of 922 workers who worked as creosote impregnators and were exposed to creosote regularly was evaluated by Karlehagen et al. (1992). SIRs were calculated from data obtained from the cancer registries in Sweden and Norway, the countries where the creosote impregnation plants were located. The period of follow-up was 27 years for the Swedish workers and 34 years for the Norwegian workers. The exposures were poorly characterized; however, the authors noted that all of the workers were exposed to naphthalene, a component of creosote. A marginally statistically significant increase in the incidence of nonmelanoma skin cancer was reported (9 cases observed versus 3.79 expected; SIR = 2.37), with most of the cases (6) reported in the Swedish workers. The authors noted that comparisons were made to national cancer rates, which could have introduced bias into the study because the base risk of cancer differed between urban and rural areas where the plants were located. The authors further noted that the workers worked partly outdoors and were exposed to UV light, which likely contributed to the incidence of skin cancer.

A.1.5 Summary of Occupational Studies

A characteristic common to occupations where an increased incidence of skin cancer was associated with PAH exposure was concomitant acute and chronic skin irritation. This irritation

took the form of burns (tar or pitch burn), erythema and edema, dermatitis, folliculitis, pigmentation, warts, hyperplasia (shagren skin), and ulceration. Additionally, the working conditions encountered were often hot, inducing perspiration, thus leading to chapping of the skin due to friction resulting from clothing. Hoffman (1928) concluded that irritation played a major role in the carcinogenic process leading to scrotal tumors in cotton mule spinners. Fisher (1953) found a significant correlation between coal tar-induced erythema and the occurrence of tar warts in tar distillation workers. Lueke (1907) reported that the skin of carbon workers became chronically inflamed, followed by moist eczema accompanied by inflamed hair follicles, acne form eruptions, and the formation of crust and scales. Ross (1948) reported that tar and pitch burns, erythema, folliculitis, acne, comedones, dermatitis, hyperplasia and melanosis were all present in tar and pitch workers. Waldron et al. (1984) noted that in one of the cases of scrotal cancer in an individual with no known exposure to PAHs, poor hygiene was a likely contributor in the development of cancer. Lastly, all of the early epidemiology studies commented on the irritating effects of soot, coal tar, paraffin, pitch, and oils to skin, as well as mechanical irritation resulting from scratching warts that are predisposed to itch.

In addition to the direct irritating effects of coal tar and pitch, exposure to the volatile and semi-volatile chemicals present in coal tar could lead to a photosensitization, thus, increasing the erythema induced by sunlight. Approximately 90% of all skin tumors identified in workers at coke ovens, coal gasification facilities, and coal tar distilleries were located on the head, neck, arms, or hands (Heller 1930). These were also the areas associated with increased skin cancer resulting from exposure to UV light (Slaper and van der Leun 1987), which is important because the potential contribution of UV light to skin cancer incidence was not considered in the occupational studies reviewed.

In summary, various studies over the last 200 years have illustrated the occurrence of skin cancer in workers employed in occupations where the potential for exposure to PAHs was high. In most instances, however, the prevalence of skin cancer in a particular occupation could also be linked to some characteristic that facilitated the formation of tumors, such as irritation, exposure to UV light, or skin conditions resulting from poor hygiene (ATSDR 1996).

REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). 1996. Toxicological Profile for Wood Creosote, Coal Tar Creosote, Coal Tar, Coal Tar Pitch, and Coal Tar Pitch Volatiles. ATSDR, Atlanta, Georgia. August 1996.

Ball CB. 1885. Tar cancer. Irish J Med Sci 80:318-321.

Bell J. 1876. Paraffin epithelioma of the scrotum. Edinburgh Med J (as cited in Butlin 1892, Lecture III).

Butlin HT. 1892. Cancer of the scrotum in chimney-sweeps and others. Lecture I. Secondary cancer without primary cancer. Br Med J 1:1341-1346.

Butlin HT. 1892. Cancer of the scrotum in chimney-sweeps and others. Lecture II. Why foreign sweeps do not suffer from scrotal cancer. Br Med J 2:1-6.

Butlin HT. 1892. Cancer of the scrotum in chimney-sweeps and others. Lecture III. Tar and paraffin cancer. Br Med J 2:66-71.

Evanoff BA, Gustavsson P, Hogstedt C. 1993. Mortality and incidence of cancer in a cohort of Swedish chimney sweeps: an extended follow up study. Br J Ind Med 50:450-459.

Fisher REW. 1953. Occupational skin cancer in a group of tar workers. Arch Ind Hyg 7:12-18.

Hammond EC, Selikoff IJ, Lawther PL, Seidman H. 1976. Inhalation of benzpyrene and cancer in man. Ann NY Acad Sci 271:116-124.

Heller I. 1930. Occupational cancers. J Ind Hyg 12:169-197.

Henry SA. 1937. The study of fatal cases of cancer of the scrotum from 1911 to 1935 in relation to occupation, with special reference to chimney sweeping and cotton mule spinning. Am J Cancer 31:28-57.

Henry SA. 1947. Occupational cutaneous cancer attributable to certain chemicals in industry. Br Med Bull 4:389-401 (As cited in IARC 1984).

Hoffman FL. 1928. Mule-spinners' cancer. US Monthly Labor Rev 27:27-45.

IARC. 1984. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol 34. Polynuclear Aromatic Compounds, Part 3. Industrial Exposures in Aluminum Production, Coal Gasification, Coke Production, and Iron and Steel Founding. International Agency for Research on Cancer, Lyon, France.

Karlehagen S, Andersen A, Ohlson C-G. 1992. Cancer incidence among creosote-exposed workers. Scand J Work Environ Health 18:26-29.

Letzel S, Drexler H. 1998. Occupationally related tumors in tar refinery workers. J Am Acad Dermatol 39:712-720.

Lueke AW. 1907. Epithelioma in carbon workers. Cleveland Med J 6:199-202.

Pott. 1775. (As cited in Butlin 1982, Lecture I).

Rønneberg A, Andersen A. 1995. Mortality and cancer morbidity in workers from an aluminum smelter with prebaked carbon anodes–part II. Cancer morbidity. Occup Environ Med 52:250-254.

Ross P. 1948. Occupational skin lesions due to pitch and tar. Br Med J 2:369-374.

Schamberg JF. 1910. Cancer in tar workers. J Cutan Dis 28:644 (As cited in Heller 1930).

Sexton RJ. 1960. The hazards to health in the hydrogenation of coal. Arch Environ Health 1:208-231.

Sherson D, Svane O, Lynge E. 1991. Cancer incidence among foundry workers in Denmark. Arch Environ health 46:75-81.

Slaper H, van der Leun JC. 1987. Human exposure to ultraviolet radiation: quantitative modelling of skin cancer incidence. In: Passchier WF, Bosnjakovic BFM, eds. Human Exposure to Ultraviolet Radiation: Risks and Regulations. Elsevier Science Publishers, New York. pp. 5-20.

Spinelli JJ, Band PR, Svirchev LM, Gallagher RP. 1991. Mortality and cancer incidence in aluminum reduction plant workers. J Occup Med 33:1150-1155.

Volkmann. 1875. On tar, paraffin, and soot cancer (chimney-sweeps' cancer). Beitrage zur Chirugie (As cited in Butlin 1892, Lecture III).

Waldron HA, Waterhouse HA, Tessema N. 1984. Scrotal cancer in the West Midlands 1936-76. Br J Ind Med 41:437-444.

Wood HB. 1929. Paraffin not productive of cancer. J Cancer Res 13:97 (As cited in Heller 1930).

Table A-1
Summary of Occupational Non-Melanoma Skin Cancer (a)

Study	Cases	Comments
Exposure to Soot		
Henry 1937	103	Chimney sweep was listed as the occupation for 103 deaths out of a total of 1,487 deaths resulting from scrotal cancer between the years 1911 and 1935 based on records kept by the Register General for England and Wales.
Waldron et al. 1984	7	The study evaluated the Cancer Registry entries made between 1936 and 1976 for the West Midland's Area in England. The follow-up period was at minimum 5 years. The incidence of scrotal cancer in sweeps represented only 2% of the 344 cases of scrotal cancer identified in the study. Scrotal cancer incidence in individuals with no known exposure to PAHs represented 16.6% of the total cases.
Evanoff et al. 1993	4	A case control study on 5,542 chimney sweeps employed from 1958-1987 in Sweden found only four cases of nonmelanoma skin cancer as opposed to an expected value of 6.5.
Exposure to Coal Tar and Pitch Workers in the paraffin, tar distillation, coke production, coke oven workers, hydrogenation of coal, gas-works, aluminum plant workers, carbon, patent fuel, asphalt, and roofing industries		
Volkman 1875 [1]	3	Case reports on individuals employed in the paraffin industry
Bell 1876 [1]	2	Case reports on individuals employed in the paraffin industry
Ball 1885 [1]	2	Case reports on individuals employed at a coal tar distillery
Lueke 1907	3	Case reports on individuals employed in the carbon industry
Ross 1948	358 91	The number of cases of skin cancer in individuals employed at coal tar distilleries reported to the Chief Inspector of Factories in Britain. The number of cases of skin cancer in individuals employed in the gas-works industry reported to the Chief Inspector of Factories in Britain.
Hoffman 1928	7 11	Seven deaths from scrotal cancer were reported in a population of 1,150 patent fuel workers in England and Wales between 1911 and 1920. Data were taken from death certificates and census data for 1911. Eleven deaths from scrotal cancer were reported in a population of 54,447 gas-works employees in England and Wales between 1911 and 1920. Data were taken from death certificates and census data for 1911.
Henry 1947 [2]	2,229	Of 3,753 total skin cancer cases reported to the Chief Inspector of Factories in Britain, 2,229 were thought to be the result of exposure to coal tar or pitch. However, no attempts were made to illustrate numbers by occupation.

Table A-1 (continued)

Study	Cases	Comments
Heller 1930		All identified cases of skin cancer at the following industries and hospitals between 1920 and 1928 based on interviews and records.
	0	No skin cancer was reported in a cohort of 300 workers employed at ten coal tar distillation facilities operated by the American Tar Products Company.
	0	No skin cancer was reported in workers employed at coal tar distillation facilities operated by the Chicago Gas Company.
	0	No skin cancer was reported in workers employed at coal tar distillation facilities or coke ovens operated by the Chicago By-Products Company
	0	No skin cancer was reported in workers employed at various coal tar distillation facilities primarily producing pitch in Chicago.
	19	Nineteen cases of skin cancer treated at Memorial Hospital, New York, and the New York Skin and Cancer Hospital between 1920 and 1928 were reported to be the result of exposure to coal tar or pitch; four roofers, two coal tar distillery workers, one coke oven worker, 12 gas works employees.
	21	Three scrotal tumors, seven tumors on the head or neck, and 11 tumors on the hand, wrist or arm were reported in an unspecified number of workers employed at a Cleveland industrial plant that used large quantities of pitch
Wood 1929 [3]	0	No cases of skin cancer were reported for 88 coal tar handlers at various gas-works or coke oven facilities in Pennsylvania.
	0	No cases of skin cancer were reported for 750 workers employed by the Philadelphia Gas Company.
	0	No cases of skin cancer were reported for 500 workers employed at the Barrett distillation plant.
Schamberg 1910 [3]	5	Five cases of skin cancer, one located on the scrotum and four located on the wrist or hand, were reported in workers exposed to coal tar for 13-55 years at a roofing paper manufacturing plant. The scrotal cancer was preceded by preliminary skin irritation.
Sexton 1960	5	Four cases of squamous cell carcinoma located on the head, hand, or buttocks, and one case of basal cell carcinoma were reported in a cohort of 359 workers employed at a coal hydrogenation plant. Latency period ranged from 9 to 116 months. The author reported that incidence rate was significantly increased when compared to the background incidence rate of skin cancer reported by Dorn and Cutler.

Table A-1 (continued)

Study	Cases	Comments
Waldron et al. 1984	27	The study evaluated the Cancer Registry entries made between 1936 and 1976 for the West Midland's Area in England. The follow-up period was at minimum 5 years. The incidence of scrotal cancer in individuals exposed to tar or pitch represented only 7.9% of the 344 cases of scrotal cancer identified in the study. Scrotal cancer incidence in individuals with no known exposure to PAHs represented 16.6% of the total cases.
Hammond et al. 1976	5	Reported on the deaths from skin cancer in roofers belonging to the United Slate, Tile and Composition Roofers, Damp and Waterproof Workers Association Union. In union members of 9-19 years, two incidences of cancer were observed as opposed to 0.43 expected (SMR = 4.65). In union members of 20+ years, three cases of skin cancer were noted as opposed to 0.75 expected (SMR = 4.00). Exposure data were presented, and ranged between 14 µg BaP/m^3 in the roof tarring area to 6,000 µg/m3 in the coal tar roofing kettle area. However, the cases of skin cancer were not identified by job category.
Letzel and Drexler 1998	249	The cohort consisted of individuals recognized as having occupationally induced disease between 1946 and 1996. Although the authors claim that the role of UV light in this cohort is questionable, more than 90% of the SCCs and BCCs were located in sun-exposed areas. Thus, there is a high probability that UV light did contribute to the formation of tumors in this cohort.
Sherson et al. 1991	65	The standard morbidity ratio (SMR) for nonmelanoma skin cancer was 0.93 (65 cases observed/69.86 cases expected), and thus, the authors reported that there was no significant increase in the incidence of skin cancer in this cohort of foundry workers, when compared to the general population.
Spinelli et al.. 1991	38	The SIR for nonmelanoma skin cancer was only 0.51 (38 cases observed/74.31 cases expected = 0.51) in this cohort of aluminum workers exposed to coal tar pitch volatiles.
Ronneberg and Andersen 1995	8	The Standardized Incidence Ratio (SIR = observed/expected) for skin cancer in workers employed for less than 3 years was 3.09. However, for workers employed for 3 or more years, the SIR for skin cancer was only 0.79.
Exposure to Creosote		
Karlehagen et al. 1992	9	A marginally statistically significant increase in the incidence of nonmelanoma skin cancer was reported (9 cases observed versus 3.79 expected; SIR = 2.37). As noted by the authors comparisons were made to national cancer rates, which could have introduced bias into the study because the base risk of cancer differed between urban and rural areas where the plants were located, and the workers worked partly outdoors and were exposed to UV light, which likely contributed to the incidence of skin cancer.

Table A-1 (continued)

Study	Cases	Comments
Exposure to mineral and cutting oils		
Hoffman 1928	100	Number of deaths occurring in a cohort of 74,625 mule spinners between 1911 and 1920 based on death certificates and census data for England and Wales.
Henry 1937	449	Number of deaths occurring in mule spinners reported between 1911 and 1935. Approximately 76% of the cancer cases involved the scrotum.
Heller 1930	5 6	A total of five deaths from skin cancer was reported in mule spinners for the areas of Fall River and New Bedford in the U.S. Six cases of scrotal cancer out of a total of 25 cases treated from 1887-1928 at Massachusetts General Hospital, Huntington Memorial Hospital, and City Hospital, Boston
Waldron et al. 1984	213	The study evaluated the Cancer Registry entries made between 1936 and 1976 for the West Midland's Area in England. The follow-up period was at minimum 5 years. The incidence of scrotal cancer in individuals exposed to mineral oils, including the cotton mule spinners, represented 61.9%% of the 344 cases of scrotal cancer identified in the study. Scrotal cancer incidence in individuals with no known exposure to PAHs represented 16.6% of the total cases.

[a] All skin cancers were nonmelanoma unless otherwise stated in the comments for each study.
[1] Data were extracted from the review on scrotal cancer presented by Butlin (1892).
[2] Data were extracted from IARC (1984).
[3] Data were extracted from the review on skin cancer presented by Heller (1930).

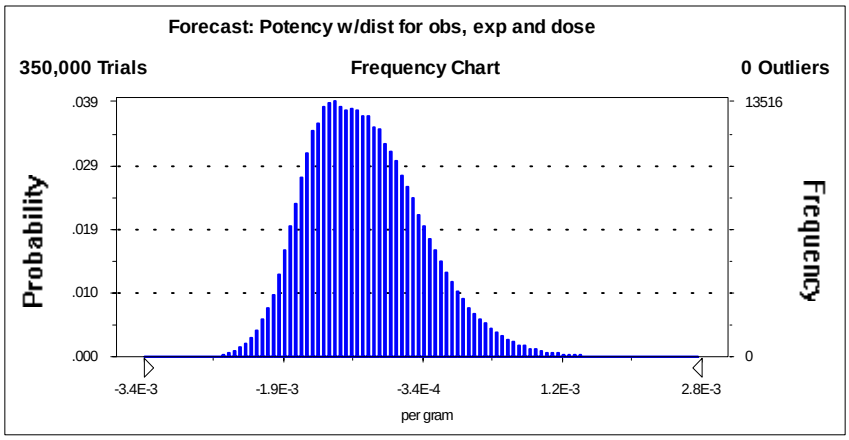
Appendix B
Estimate of Cumulative Exposure to Coal Tar for Pittelkow Cohort

Crystal Ball Report
Simulation started on 7/11/00 at 16:42:18
Simulation stopped on 7/14/00 at 12:25:27

Forecast: Potency w/dist for obs, exp and dose **Cell: C274**

Summary:
Display Range is from -3.4E-3 to 2.8E-3 per gram
Entire Range is from -3.4E-3 to 2.8E-3 per gram
After 350,000 Trials, the Std. Error of the Mean is 1.0E-6

Statistics:	Value
Trials	350000
Mean	-9.6E-04
Median	-1.0E-03
Mode	---
Standard Deviation	6.4E-04
Variance	4.1E-07
Skewness	5.50E-01
Kurtosis	3.41E+00
Coeff. of Variability	-6.69E-01
Range Minimum	-3.4E-03
Range Maximum	2.8E-03
Range Width	6.2E-03
Mean Std. Error	1.09E-06



Forecast: Potency w/dist for obs, exp and dose (cont'd) **Cell: C274**

Percentiles:

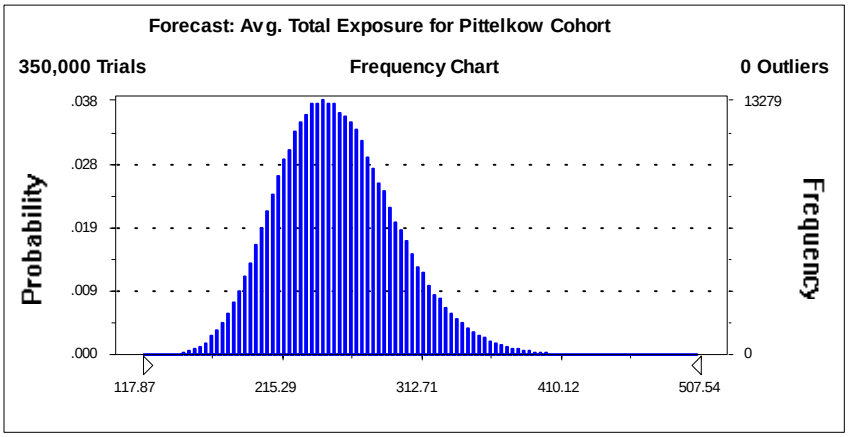
Percentile	per gram
0%	-3.4E-03
5%	-1.9E-03
10%	-1.7E-03
15%	-1.6E-03
20%	-1.5E-03
25%	-1.4E-03
30%	-1.4E-03
35%	-1.3E-03
40%	-1.2E-03
45%	-1.1E-03
50%	-1.0E-03
55%	-9.4E-04
60%	-8.5E-04
65%	-7.6E-04
70%	-6.7E-04
75%	-5.6E-04
80%	-4.4E-04
85%	-2.9E-04
90%	-1.1E-04
95%	1.8E-04
100%	2.8E-03

End of Forecast

Forecast: Avg. Total Exposure for Pittelkow Cohort **Cell: C271**

Summary:
Display Range is from 117.87 to 507.54
Entire Range is from 117.87 to 507.54
After 350,000 Trials, the Std. Error of the Mean is 0.07

Statistics:	Value
Trials	350000
Mean	254.46
Median	251.54
Mode	---
Standard Deviation	41.67
Variance	1,736.64
Skewness	0.41
Kurtosis	3.25
Coeff. of Variability	0.16
Range Minimum	117.87
Range Maximum	507.54
Range Width	389.66
Mean Std. Error	0.07



Forecast: Avg. Total Exposure for Pittelkow Cohort (cont'd) Cell: C271

Percentiles:

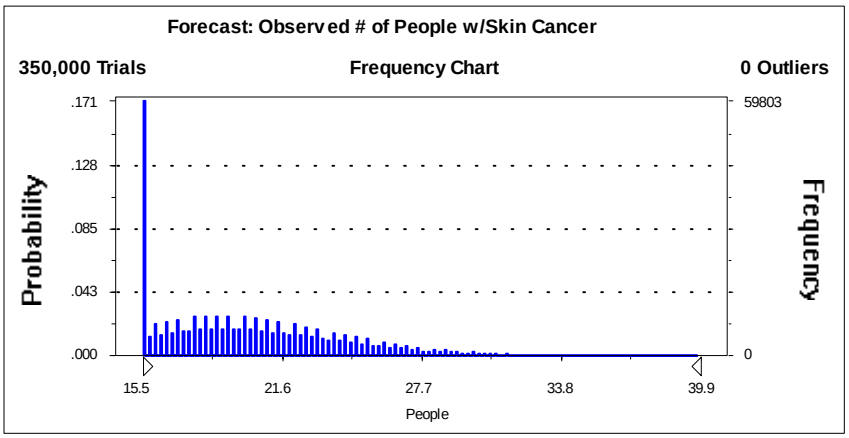
Percentile	Value
0%	117.87
5%	191.18
10%	203.27
15%	211.81
20%	218.79
25%	225.02
30%	230.68
35%	236.04
40%	241.24
45%	246.40
50%	251.54
55%	256.86
60%	262.32
65%	267.99
70%	274.03
75%	280.78
80%	288.42
85%	297.64
90%	309.37
95%	327.67
100%	507.54

End of Forecast

Forecast: Observed # of People w/Skin Cancer **Cell: C273**

Summary:
Display Range is from 15.5 to 39.9 People
Entire Range is from 15.5 to 39.9 People
After 350,000 Trials, the Std. Error of the Mean is 0.0

Statistics:	Value
Trials	350000
Mean	20.3
Median	19.7
Mode	15.5
Standard Deviation	4.1
Variance	16.5
Skewness	0.84
Kurtosis	3.43
Coeff. of Variability	0.20
Range Minimum	15.5
Range Maximum	39.9
Range Width	24.4
Mean Std. Error	0.01



Forecast: Observed # of People w/Skin Cancer (cont'd) **Cell: C273**

Percentiles:

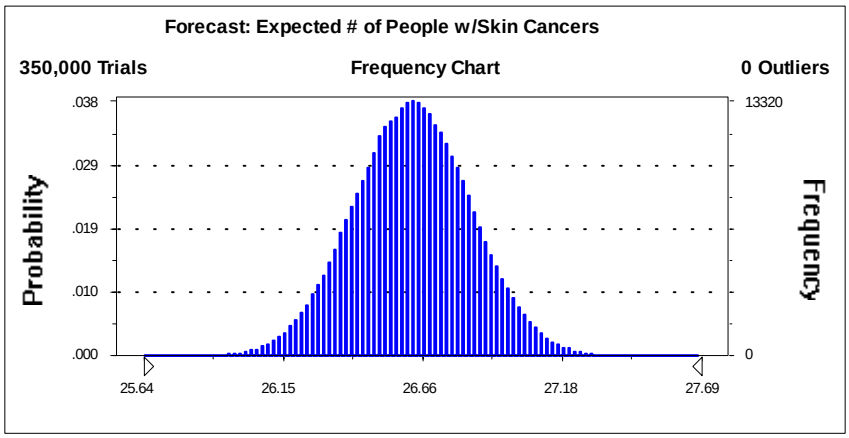
Percentile	People
0%	15.5
5%	15.5
10%	15.5
15%	15.5
20%	16.2
25%	16.8
30%	17.4
35%	18.0
40%	18.6
45%	19.1
50%	19.7
55%	20.2
60%	20.8
65%	21.4
70%	22.1
75%	22.8
80%	23.6
85%	24.6
90%	25.9
95%	27.9
100%	39.9

End of Forecast

Forecast: Expected # of People w/Skin Cancers **Cell: C272**

Summary:
Display Range is from 25.64 to 27.69
Entire Range is from 25.64 to 27.69
After 350,000 Trials, the Std. Error of the Mean is 0.00

Statistics:	Value
Trials	350000
Mean	26.63
Median	26.63
Mode	---
Standard Deviation	0.22
Variance	0.05
Skewness	0.00
Kurtosis	3.00
Coeff. of Variability	0.01
Range Minimum	25.64
Range Maximum	27.69
Range Width	2.05
Mean Std. Error	0.00



Forecast: Expected # of People w/Skin Cancers (cont'd) **Cell: C272**

Percentiles:

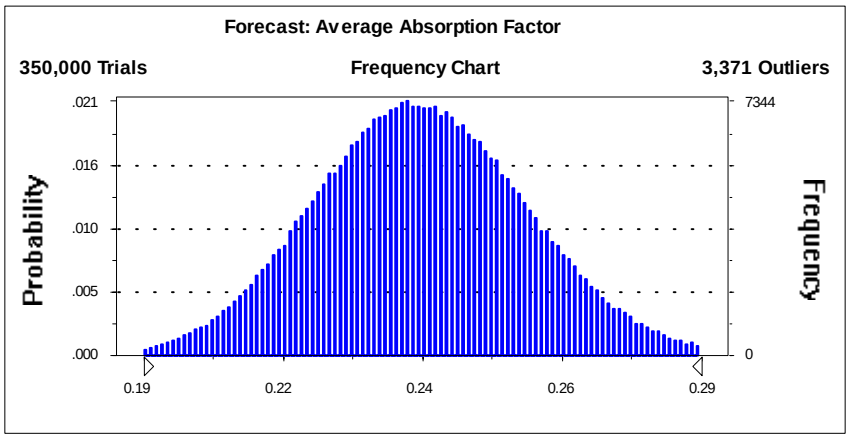
Percentile	Value
0%	25.64
5%	26.27
10%	26.35
15%	26.41
20%	26.45
25%	26.48
30%	26.52
35%	26.55
40%	26.58
45%	26.60
50%	26.63
55%	26.66
60%	26.69
65%	26.71
70%	26.74
75%	26.78
80%	26.81
85%	26.85
90%	26.91
95%	26.99
100%	27.69

End of Forecast

Forecast: Average Absorption Factor **Cell: H271**

Summary:
Display Range is from 0.19 to 0.29
Entire Range is from 0.16 to 0.33
After 350,000 Trials, the Std. Error of the Mean is 0.00

Statistics:	Value
Trials	350000
Mean	0.24
Median	0.24
Mode	0.25
Standard Deviation	0.02
Variance	0.00
Skewness	0.10
Kurtosis	3.02
Coeff. of Variability	0.08
Range Minimum	0.16
Range Maximum	0.33
Range Width	0.17
Mean Std. Error	0.00



Forecast: Average Absorption Factor (cont'd) **Cell: H271**

Percentiles:

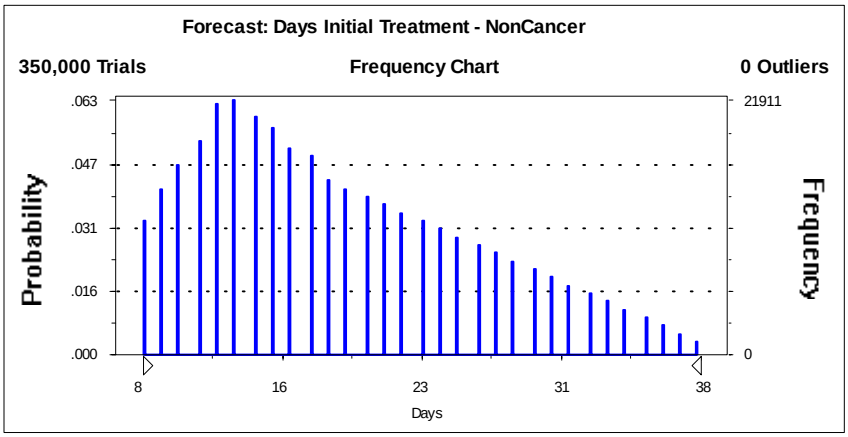
Percentile	Value
0%	0.158
5%	0.211
10%	0.217
15%	0.221
20%	0.225
25%	0.228
30%	0.230
35%	0.233
40%	0.235
45%	0.238
50%	0.240
55%	0.242
60%	0.244
65%	0.247
70%	0.249
75%	0.252
80%	0.255
85%	0.259
90%	0.264
95%	0.270
100%	0.328

End of Forecast

Forecast: Days Initial Treatment - NonCancer **Cell: U10**

Summary:
Display Range is from 8 to 38 Days
Entire Range is from 8 to 38 Days
After 350,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	350000
Mean	19
Median	17
Mode	13
Standard Deviation	7
Variance	53
Skewness	0.58
Kurtosis	2.45
Coeff. of Variability	0.39
Range Minimum	8
Range Maximum	38
Range Width	30
Mean Std. Error	0.01



Forecast: Days Initial Treatment - NonCancer (cont'd) **Cell: U10**

Percentiles:

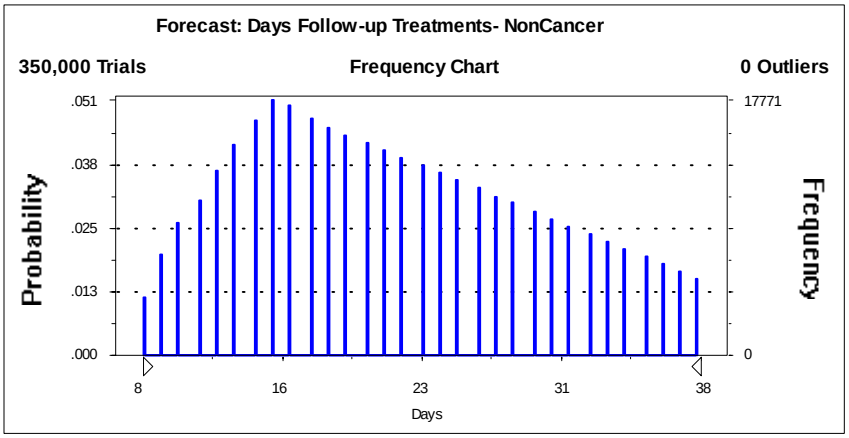
Percentile	Days
0%	8
5%	9
10%	10
15%	11
20%	12
25%	13
30%	14
35%	14
40%	15
45%	16
50%	17
55%	18
60%	20
65%	21
70%	22
75%	24
80%	25
85%	27
90%	30
95%	33
100%	38

End of Forecast

Forecast: Days Follow-up Treatments- NonCancer **Cell: V10**

Summary:
Display Range is from 8 to 38 Days
Entire Range is from 8 to 38 Days
After 350,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	350000
Mean	22
Median	21
Mode	15
Standard Deviation	8
Variance	61
Skewness	0.31
Kurtosis	2.11
Coeff. of Variability	0.36
Range Minimum	8
Range Maximum	38
Range Width	30
Mean Std. Error	0.01



Forecast: Days Follow-up Treatments- NonCancer (cont'd) **Cell: V10**

Percentiles:

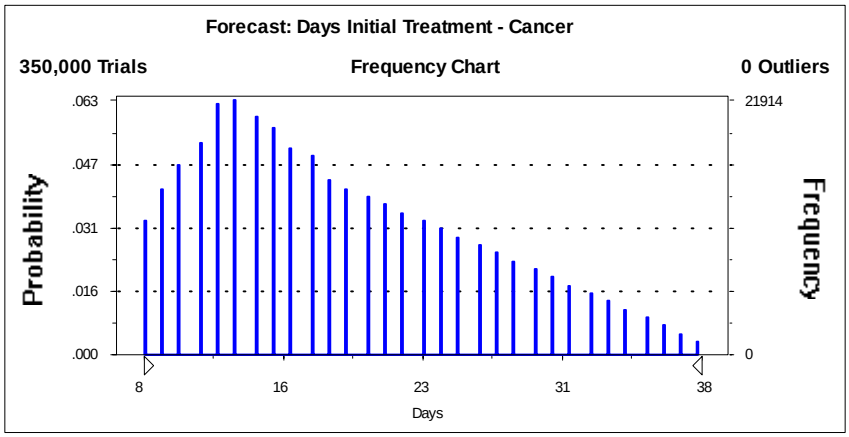
Percentile	Days
0%	8
5%	10
10%	12
15%	13
20%	14
25%	15
30%	16
35%	17
40%	18
45%	19
50%	21
55%	22
60%	23
65%	25
70%	26
75%	28
80%	29
85%	31
90%	33
95%	36
100%	38

End of Forecast

Forecast: Days Initial Treatment - Cancer **Cell: U251**

Summary:
Display Range is from 8 to 38 Days
Entire Range is from 8 to 38 Days
After 350,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	350000
Mean	19
Median	17
Mode	13
Standard Deviation	7
Variance	53
Skewness	0.58
Kurtosis	2.45
Coeff. of Variability	0.39
Range Minimum	8
Range Maximum	38
Range Width	30
Mean Std. Error	0.01



Forecast: Days Initial Treatment - Cancer (cont'd) **Cell: U251**

Percentiles:

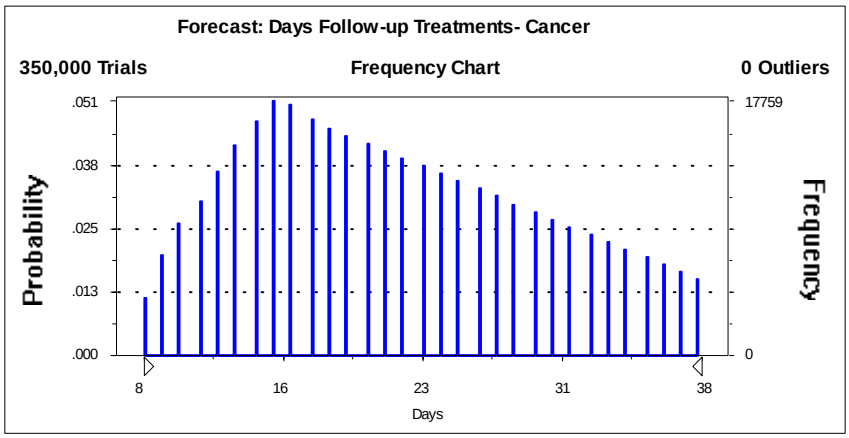
Percentile	Days
0%	8
5%	9
10%	10
15%	11
20%	12
25%	13
30%	14
35%	14
40%	15
45%	16
50%	17
55%	18
60%	20
65%	21
70%	22
75%	24
80%	25
85%	27
90%	30
95%	33
100%	38

End of Forecast

Forecast: Days Follow-up Treatments- Cancer **Cell: V251**

Summary:
Display Range is from 8 to 38 Days
Entire Range is from 8 to 38 Days
After 350,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	350000
Mean	22
Median	21
Mode	15
Standard Deviation	8
Variance	61
Skewness	0.31
Kurtosis	2.11
Coeff. of Variability	0.36
Range Minimum	8
Range Maximum	38
Range Width	30
Mean Std. Error	0.01



Forecast: Days Follow-up Treatments- Cancer (cont'd) **Cell: V251**

Percentiles:

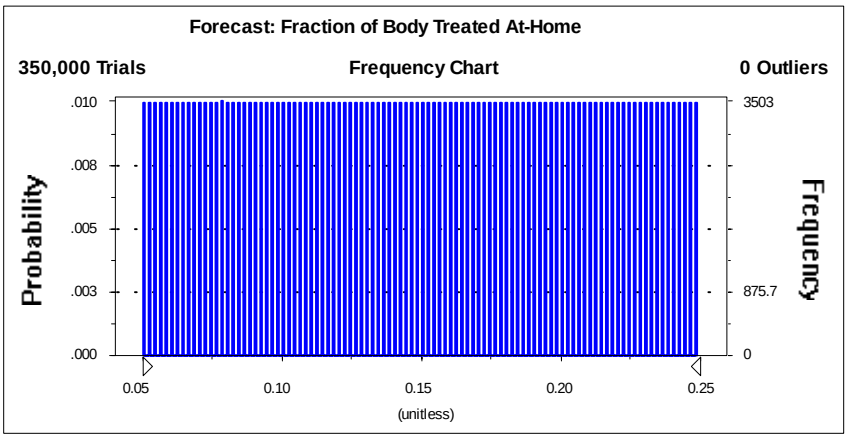
Percentile	Days
0%	8
5%	10
10%	12
15%	13
20%	14
25%	15
30%	16
35%	17
40%	18
45%	19
50%	21
55%	22
60%	23
65%	25
70%	26
75%	28
80%	29
85%	31
90%	33
95%	36
100%	38

End of Forecast

Forecast: Fraction of Body Treated At-Home **Cell: Q10**

Summary:
Display Range is from 0.05 to 0.25 (unitless)
Entire Range is from 0.05 to 0.25 (unitless)
After 350,000 Trials, the Std. Error of the Mean is 0.00

Statistics:	Value
Trials	350000
Mean	0.15
Median	0.15
Mode	---
Standard Deviation	0.06
Variance	0.00
Skewness	0.00
Kurtosis	1.80
Coeff. of Variability	0.38
Range Minimum	0.05
Range Maximum	0.25
Range Width	0.20
Mean Std. Error	0.00



Forecast: Fraction of Body Treated At-Home (cont'd) **Cell: Q10**

Percentiles:

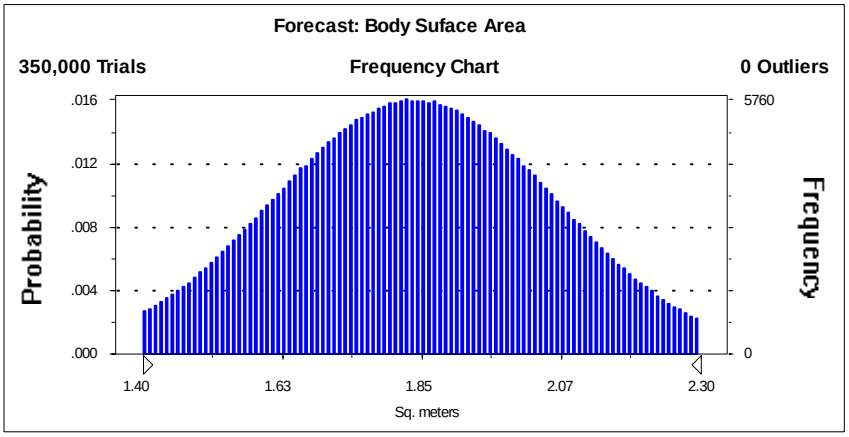
Percentile	(unitless)
0%	0.05
5%	0.06
10%	0.07
15%	0.08
20%	0.09
25%	0.10
30%	0.11
35%	0.12
40%	0.13
45%	0.14
50%	0.15
55%	0.16
60%	0.17
65%	0.18
70%	0.19
75%	0.20
80%	0.21
85%	0.22
90%	0.23
95%	0.24
100%	0.25

End of Forecast

Forecast: Body Suface Area **Cell: P10**

Summary:
Display Range is from 1.40 to 2.30 Sq. meters
Entire Range is from 1.40 to 2.30 Sq. meters
After 350,000 Trials, the Std. Error of the Mean is 0.00

Statistics:	Value
Trials	350000
Mean	1.84
Median	1.84
Mode	---
Standard Deviation	0.20
Variance	0.04
Skewness	0.02
Kurtosis	2.34
Coeff. of Variability	0.11
Range Minimum	1.40
Range Maximum	2.30
Range Width	0.90
Mean Std. Error	0.00



Forecast: Body Suface Area (cont'd) **Cell: P10**

Percentiles:

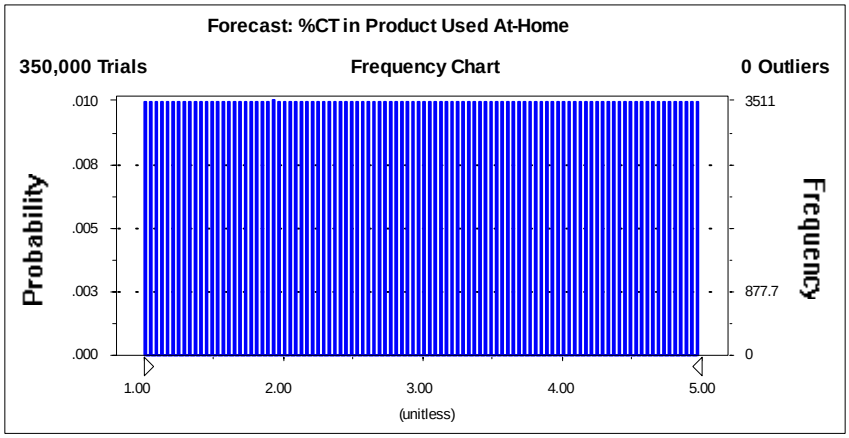
Percentile	Sq. meters
0%	1.40
5%	1.51
10%	1.57
15%	1.62
20%	1.66
25%	1.70
30%	1.73
35%	1.76
40%	1.79
45%	1.81
50%	1.84
55%	1.87
60%	1.90
65%	1.93
70%	1.96
75%	1.99
80%	2.02
85%	2.06
90%	2.11
95%	2.18
100%	2.30

End of Forecast

Forecast: %CT in Product Used At-Home **Cell: R10**

Summary:
Display Range is from 1.00 to 5.00 (unitless)
Entire Range is from 1.00 to 5.00 (unitless)
After 350,000 Trials, the Std. Error of the Mean is 0.00

Statistics:	Value
Trials	350000
Mean	3.00
Median	3.00
Mode	---
Standard Deviation	1.15
Variance	1.33
Skewness	0.00
Kurtosis	1.80
Coeff. of Variability	0.38
Range Minimum	1.00
Range Maximum	5.00
Range Width	4.00
Mean Std. Error	0.00



Forecast: %CT in Product Used At-Home (cont'd) **Cell: R10**

Percentiles:

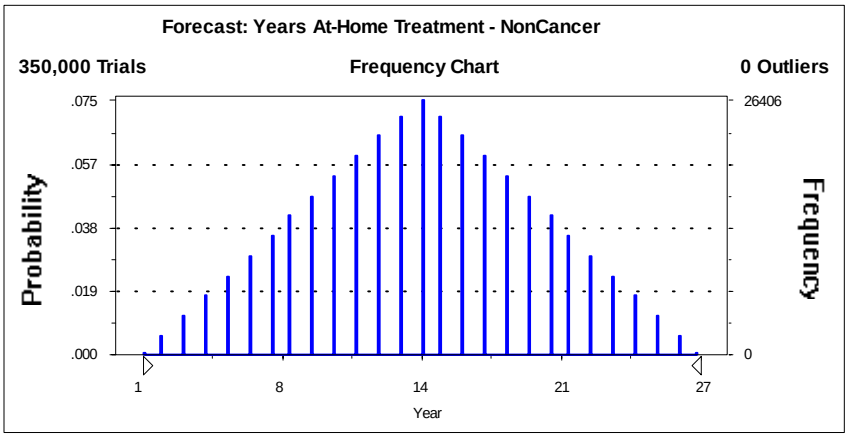
Percentile	(unitless)
0%	1.0
5%	1.2
10%	1.4
15%	1.6
20%	1.8
25%	2.0
30%	2.2
35%	2.4
40%	2.6
45%	2.8
50%	3.0
55%	3.2
60%	3.4
65%	3.6
70%	3.8
75%	4.0
80%	4.2
85%	4.4
90%	4.6
95%	4.8
100%	5.0

End of Forecast

Forecast: Years At-Home Treatment - NonCancer **Cell: T10**

Summary:
Display Range is from 1 to 27 Year
Entire Range is from 1 to 27 Year
After 350,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	350000
Mean	14
Median	14
Mode	14
Standard Deviation	5
Variance	28
Skewness	0.00
Kurtosis	2.40
Coeff. of Variability	0.38
Range Minimum	1
Range Maximum	27
Range Width	26
Mean Std. Error	0.01



Forecast: Years At-Home Treatment - NonCancer (cont'd) **Cell: T10**

Percentiles:

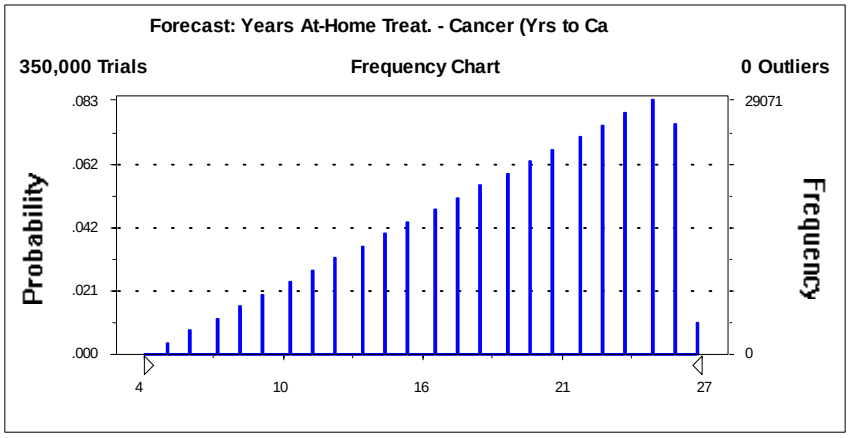
Percentile	Year
0%	1
5%	5
10%	7
15%	8
20%	9
25%	10
30%	11
35%	12
40%	13
45%	13
50%	14
55%	15
60%	15
65%	16
70%	17
75%	18
80%	19
85%	20
90%	21
95%	23
100%	27

End of Forecast

Forecast: Years At-Home Treat. - Cancer (Yrs to Ca **Cell: T251**

Summary:
Display Range is from 4 to 27
Entire Range is from 4 to 27
After 350,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	350000
Mean	19
Median	20
Mode	25
Standard Deviation	5
Variance	28
Skewness	-0.56
Kurtosis	2.40
Coeff. of Variability	0.28
Range Minimum	4
Range Maximum	27
Range Width	23
Mean Std. Error	0.01



Forecast: Years At-Home Treat. - Cancer (Yrs to Ca (cont'd) Cell: T251

Percentiles:

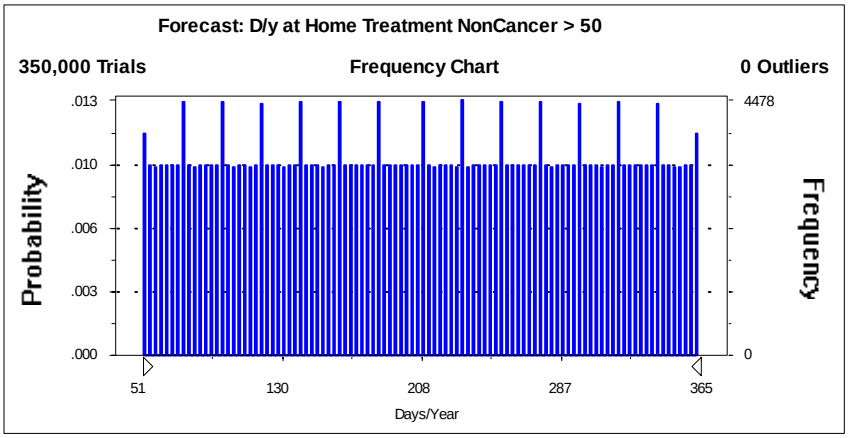
Percentile	Value
0%	4
5%	9
10%	11
15%	13
20%	14
25%	15
30%	16
35%	17
40%	18
45%	19
50%	20
55%	21
60%	21
65%	22
70%	23
75%	23
80%	24
85%	25
90%	25
95%	26
100%	27

End of Forecast

Forecast: D/y at Home Treatment NonCancer > 50 **Cell: W10**

Summary:
Display Range is from 51 to 365 Days/Year
Entire Range is from 51 to 365 Days/Year
After 350,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	350000
Mean	208
Median	208
Mode	169
Standard Deviation	91
Variance	8,217
Skewness	0.00
Kurtosis	1.80
Coeff. of Variability	0.44
Range Minimum	51
Range Maximum	365
Range Width	314
Mean Std. Error	0.15



Forecast: D/y at Home Treatment NonCancer > 50 (cont'd) **Cell: W10**

Percentiles:

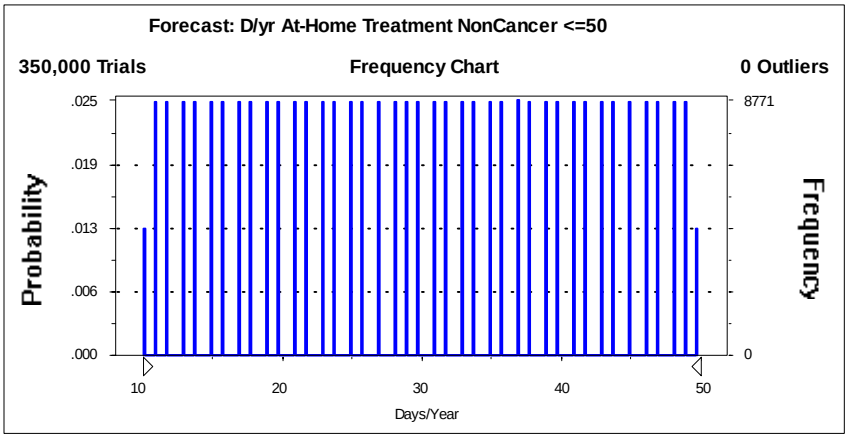
Percentile	Days/Year
0%	51
5%	67
10%	82
15%	98
20%	114
25%	129
30%	145
35%	161
40%	177
45%	192
50%	208
55%	224
60%	239
65%	255
70%	271
75%	286
80%	302
85%	318
90%	334
95%	349
100%	365

End of Forecast

Forecast: D/yr At-Home Treatment NonCancer <=50 **Cell: W115**

Summary:
Display Range is from 10 to 50 Days/Year
Entire Range is from 10 to 50 Days/Year
After 350,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	350000
Mean	30
Median	30
Mode	37
Standard Deviation	12
Variance	134
Skewness	0.00
Kurtosis	1.80
Coeff. of Variability	0.39
Range Minimum	10
Range Maximum	50
Range Width	40
Mean Std. Error	0.02



Forecast: D/yr At-Home Treatment NonCancer <=50 (cont'd) Cell: W115

Percentiles:

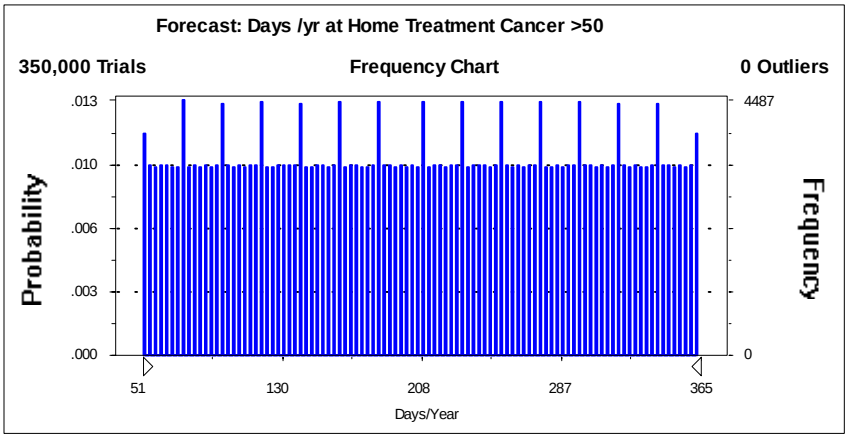
Percentile	Days/Year
0%	10
5%	12
10%	14
15%	16
20%	18
25%	20
30%	22
35%	24
40%	26
45%	28
50%	30
55%	32
60%	34
65%	36
70%	38
75%	40
80%	42
85%	44
90%	46
95%	48
100%	50

End of Forecast

Forecast: Days /yr at Home Treatment Cancer >50 **Cell: W251**

Summary:
Display Range is from 51 to 365 Days/Year
Entire Range is from 51 to 365 Days/Year
After 350,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	350000
Mean	208
Median	208
Mode	61
Standard Deviation	91
Variance	8,217
Skewness	0.00
Kurtosis	1.80
Coeff. of Variability	0.44
Range Minimum	51
Range Maximum	365
Range Width	314
Mean Std. Error	0.15



Forecast: Days /yr at Home Treatment Cancer >50 (cont'd) Cell: W251

Percentiles:

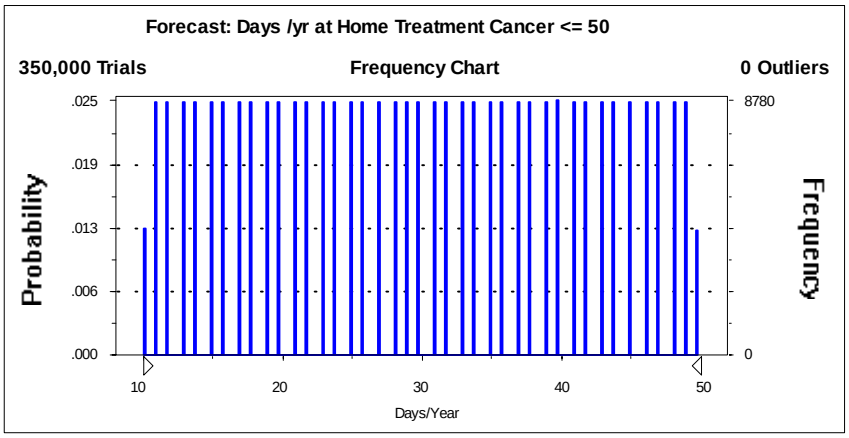
Percentile	Days/Year
0%	51
5%	67
10%	82
15%	98
20%	114
25%	129
30%	145
35%	161
40%	177
45%	192
50%	208
55%	224
60%	239
65%	255
70%	271
75%	286
80%	302
85%	318
90%	334
95%	349
100%	365

End of Forecast

Forecast: Days /yr at Home Treatment Cancer <= 50 **Cell: W258**

Summary:
Display Range is from 10 to 50 Days/Year
Entire Range is from 10 to 50 Days/Year
After 350,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	350000
Mean	30
Median	30
Mode	40
Standard Deviation	12
Variance	133
Skewness	0.00
Kurtosis	1.80
Coeff. of Variability	0.39
Range Minimum	10
Range Maximum	50
Range Width	40
Mean Std. Error	0.02



Forecast: Days /yr at Home Treatment Cancer <= 50 (cont'd) Cell: W258

Percentiles:

Percentile	Days/Year
0%	10
5%	12
10%	14
15%	16
20%	18
25%	20
30%	22
35%	24
40%	26
45%	28
50%	30
55%	32
60%	34
65%	36
70%	38
75%	40
80%	42
85%	44
90%	46
95%	48
100%	50

End of Forecast

APPENDIX C

Background Data for Calculation of Conversion Factors

Van Rooij et al. (1993a)

Two separate experiments in human volunteers were reported in this study. In one experiment, a 10% coal tar ointment in a zinc oxide and vaseline paste was applied at a dose of 2.5 mg coal tar ointment/cm^2 on a 24 cm^2/site on six sites per volunteer. Four volunteers were included in the study. Following application of the coal tar ointment, the skin was covered. After 45 minutes, the remainder of the ointment was removed and the skin site cleaned (less than 0.04% of the applied dose remained externally on the skin after cleaning). The disappearance of the mixture from the application site was monitored for up to 55 hours (8-18 measurements/site) using fiberoptics luminoscope. With the method, what is actually measured is the disappearance of chemical from the skin compartment, i.e., the amount of chemical that has already penetrated the outermost layer of skin, the stratum corneum. From these measurements, PAH absorption rate constants were estimated to be 0.026/h to 0.196/h, depending on the site of application, by assuming that the absorption of PAHs follows first order kinetics. These data were used to estimate skin absorption in workers exposed to coke oven particulates (Van Rooij et al. 1993b), as discussed below.

In the second experiment, 2.5 mg/cm^2 of a 10% coal tar ointment in a zinc oxide and vaseline base was applied to 400 cm^2 skin per volunteer (eight subjects). This was equivalent to 2 µg pyrene/cm^2. The sites were occluded with plastic and cotton covering for 6 hours, after which the site was cleaned with warm soap and water. Urinary excretion of 1-OH-P was measured 24 hours prior to exposure and for 72 hours after exposure. The amount of dermally absorbed pyrene was estimated based on the following assumptions:

1. Based on rat data from Jacob et al. (1989), it was assumed that 46% of the systemically available (absorbed) pyrene was metabolized to 1-OH-P.

2. Based on a comparison of the urinary and fecal excretion of 1-OH-P in human volunteers, it was estimated that 90% of the 1-OH-P was excreted in the urine. The 1-OH-P fecal excretion rate in volunteers did not increase after coal tar application, while urinary levels

increased 6- to 10-fold above background following dermal application of the coal tar ointment.

3. Storage of pyrene or 1-OH-P in the body is very limited, since 1-OH-P levels returned to background levels within the time course of the experiment (within 48-60 hours).

Based on these assumptions, the percent conversion of absorbed pyrene to 1-OH-P was 41% (46%*90%); therefore, the authors estimated that total dermal absorption during the 6-hour exposure period was 0.3-1.4% of the applied pyrene dose. The authors stressed that, "occlusion, due to the ointment itself and the coverage of the site with plastic, may have increased dermal PAH absorption." Absorption was also likely overestimated because of the use of rat data for the first component – metabolic conversion of pyrene to 1-OH-P. *In vitro* data on the rate of metabolism in human and rat hepatocytes demonstrated that the rat metabolizes pyrene to 1-OH-P at approximately 50% the rate for humans (Jongeneelen and Bos 1990), as discussed below.

Van Rooij et al. (1993b)

In another study conducted by Van Rooij et al. (1993b), PAH exposure in 12 coke oven workers was evaluated. Dermal exposure was estimated from exposure pads placed on six skin sites for the duration of an 8-hour work shift, daily for 5 work days. The exposure pads consisted of flexible polypropylene filters as the absorbing material. Urinary levels of 1-OH-P were measured daily during the 5-day work shift, and for 2 additional days. The authors reported that the conversion of absorbed pyrene to 1-OH-P was influenced by smoking and was 13% for nonsmokers and ranged from 18% to 49% for smokers. Several assumptions were made in this assessment.

First, and most important, the authors estimated the amount of absorbed pyrene by applying a model based on the rate of pyrene absorption from another study (Van Rooij et al. 1993a) in which a coal tar ointment in a zinc oxide and vaseline base was applied to the skin. The dermal absorption rate represents the disappearance from the surface and skin compartment with the underlying assumption that the rate limiting step is the removal from the skin

compartment rather than penetration of the stratum corneum. In using these data, the unstated assumption is two-fold:

1. The amount of pyrene adsorbed/absorbed onto/into the polyethylene filters is representative of the amount of coke oven dust that comes in sustained contact with the skin.

2. The penetration of coke oven particles occurs at the same rate as that of PAHs contained in an ointment in a vaseline base applied to the skin and covered with an occluded dressing.

Using the rate constants derived from the ointment study, the authors estimated the amount of absorption of PAHs from coke oven dust. Then, by ratioing the amount of 1-OH-P in the urine with the calculated percentage absorption, they "backed out" the conversion rates of absorbed pyrene to 1-OH-P. Use of this approach will result in an overestimate of the amount of pyrene absorbed, and, consequently, underestimate the conversion percentage of pyrene to 1-OHP in the urine. In this study, dermal absorption of coke oven particulates was calculated to be greater than 20%. Wester et al. (1990) found only about a 20-24% absorption rate, when BaP was applied in an acetone vehicle and occluded for 24 hours, which was greatly reduced when BaP was applied in a soil matrix. Similar differences in absorption have been noted between soil and nonsoil matrices by other investigators (van Schooten et al. 1997). While coke oven dust is not "bound" in a soil matrix, it is not as readily available as it would be in an acetone vehicle or in a coal tar, vaseline-based ointment. Moreover, the conversion rate of 13% for nonsmokers was based on one individual. The rate for smokers consisted of five individuals with highly variable estimates of absorption.

Van Rooij (1995)

This is a non-peer reviewed document prepared by Industox that proposed a kinetic model for dermal absorption from a shampooing event. In this paper, the percentages used for

the pyrene to 1-OH-P metabolism and the 1-OH-P to urine fraction were 75% and 50%, respectively. In this case, the 75% was based on human data using the *in vitro* study reported by Jongeneelen and Bos (1990), and the 50% was based on rat data reported by Withey et al. (1991). The resulting conversion percentage would be 37.5%.

Singh et al. (1995)

In this study, human volunteers exposed to mixtures of PAHs while working in a coal gasification plant were evaluated. The major pyrene metabolite detected was the glucuronide conjugate of 1-OH-P (1-OH-P-GlcUA). This metabolite accounted for 80-100% of the pyrene metabolites excreted in the urine of these volunteers. The levels of 1-OH-P-GlcUA in the urine ranged from 0.31 to 0.94 µg/g creatinine. When the urine was subjected to enzymatic hydrolysis using glucuronidase, which is the standard procedure in the 1-OH-P assays, the levels of 1-OH-P ranged from 0.51 to 1.17 µg/g creatinine. Hence, the total 1-OH-P metabolites in the urine is greater than 80% and approaches 100% of all pyrene metabolites excreted.

Jongeneelen and Bos (1990)

An *in vitro* study in human and rat hepatocytes evaluated the rate of pyrene metabolism to 1-OH-P. The rate of metabolism was dose dependent in both species and followed Michaelis-Menten kinetics; the K_m in humans was estimated to be 12.5 µM, while that in rats was 20.3 µM. At the lowest concentration tested, 2 µmol/l, the percentage of 1-OH-P formed after 30 minutes incubation was 30% in rats and 60% in the human hepatocytes. According to the authors, the initial reaction velocity remained constant for 60 minutes, indicating that at the end of that time at the lowest concentration it would be expected that 100% of the substrate, pyrene, would have been metabolized to 1-OH-P. Based on the total mass of pyrene available for absorption from the shampooing event, the absorbed amount of pyrene body burden would be expected to be at an ultimate concentration in the liver lower than the lowest concentration tested in this assay. Hence, metabolism of pyrene in humans is expected to be virtually complete.

References

Jacob J, Brune H, Gettbarn G, Grimmer D, Heinrich U, Mohtashamipur E, Norpoth K, Pott F, Wenzel-Hartung R. 1989. Urinary and faecal excretion of pyrene and hydroxypyrene by rats after oral, intraperitoneal intratracheal or intrapulmonary application. Cancer Lett 46:15-20

Jongeneelen F, Bos R. 1990. Excretion of pyrene and hydroxypyrene in urine. Letters to the editor. Cancer Lett 51:175-179.

Singh R, Tucek M, Maxa K, Tenglerova J, Weyand EH. 1995. A rapid and simple method for the analysis of 1-hydroxypyrene glucuronide: a potential biomarker for polycyclic aromatic hydrocarbon exposure. Carcinogenesis 16:2909-2915.

Van Rooij J. 1995. An assessment of the health risks in connection with using shampoo which contains tar. Industox Report No. IT-9422.

VanRooij JGM, DeRoos JHC, Bodelier-Bade MM, Jongeneelen FJ. 1993a. Absorption of polycyclic aromatic hydrocarbons through human skin: differences between anatomical sites and individuals. J Toxicol Environ Health 38:355-368.

Van Rooij J, Bodielier-Bade M, Jongeneelen F. 1993b. Estimation of individual dermal and respiratory uptake of polycyclic aromatic hydrocarbons in 12 coke oven workers. *Br J Ind Med* 50:632-632.

Van Schooten F-J, Moonen EJC, van der Wal L, Levels P, Kleinjans JCS. 1997. Determination of polycyclic aromatic hydrocarbons (PAH) and their metabolites in blood, feces, and urine of rats orally exposed to PAH contaminated soils. Arch Environ Contam Toxicol 33:317-322.

Wester RC, Maibach HI, Bucks DAW, Sedik L, Melendres J, Liao C, DiZio S. 1990. Percutaneous absorption of [^{14}C]DDT and [^{14}C]benzo(a)pyrene from soil. Fundam Appl Toxicol 15:510-516.

Withey JR, Law FCP, Endrenyi L. 1991. Pharmacokinetic and bioavailability of pyrene in the rat. J Toxicol Environ Health 32:429-447.

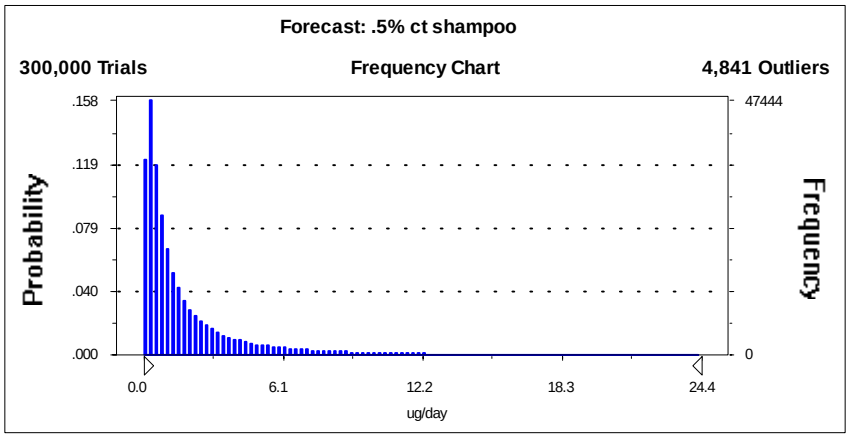
Appendix D
Estimates of Lifetime Average Daily Dose From Exposure to Coal Tar in Shampoo

Crystal Ball Report
Simulation started on 7/14/00 at 14:28:40
Simulation stopped on 7/14/00 at 14:32:09

Forecast: .5% ct shampoo **Cell: B16**

Summary:
Display Range is from 0.0 to 24.4 ug/day
Entire Range is from 0.0 to 623.4 ug/day
After 300,000 Trials, the Std. Error of the Mean is 0.0

Statistics:	Value
Trials	300000
Mean	3.0
Median	1.0
Mode	---
Standard Deviation	8.2
Variance	67.6
Skewness	15.55
Kurtosis	513.74
Coeff. of Variability	2.73
Range Minimum	0.0
Range Maximum	623.4
Range Width	623.4
Mean Std. Error	0.02



Forecast: .5% ct shampoo (cont'd) **Cell: B16**

Percentiles:

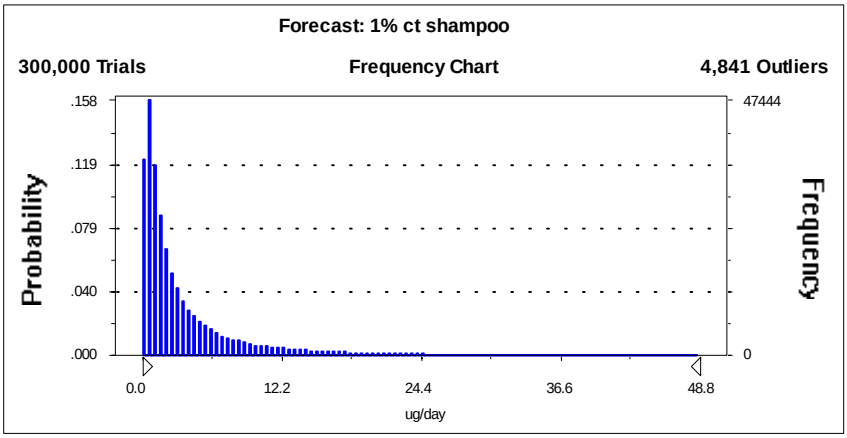
Percentile	ug/day
0%	0.0
5%	0.1
10%	0.2
15%	0.3
20%	0.4
25%	0.4
30%	0.5
35%	0.6
40%	0.7
45%	0.9
50%	1.0
55%	1.2
60%	1.4
65%	1.7
70%	2.1
75%	2.6
80%	3.4
85%	4.5
90%	6.5
95%	11.3
100%	623.4

End of Forecast

Forecast: 1% ct shampoo **Cell: B17**

Summary:
Display Range is from 0.0 to 48.8 ug/day
Entire Range is from 0.0 to 1,246.8 ug/day
After 300,000 Trials, the Std. Error of the Mean is 0.0

Statistics:	Value
Trials	300000
Mean	6.0
Median	2.1
Mode	---
Standard Deviation	16.4
Variance	270.6
Skewness	15.55
Kurtosis	513.74
Coeff. of Variability	2.73
Range Minimum	0.0
Range Maximum	1,246.8
Range Width	1,246.8
Mean Std. Error	0.03



Forecast: 1% ct shampoo (cont'd) **Cell: B17**

Percentiles:

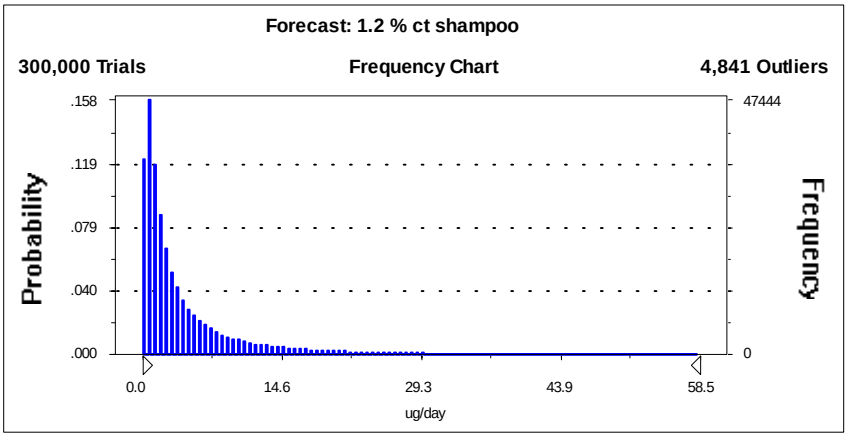
Percentile	ug/day
0%	0.0
5%	0.3
10%	0.4
15%	0.6
20%	0.7
25%	0.9
30%	1.0
35%	1.2
40%	1.5
45%	1.7
50%	2.1
55%	2.4
60%	2.9
65%	3.5
70%	4.3
75%	5.3
80%	6.7
85%	9.0
90%	13.0
95%	22.6
100%	1,246.8

End of Forecast

Forecast: 1.2 % ct shampoo **Cell: B18**

Summary:
Display Range is from 0.0 to 58.5 ug/day
Entire Range is from 0.0 to 1,496.2 ug/day
After 300,000 Trials, the Std. Error of the Mean is 0.0

Statistics:	Value
Trials	300000
Mean	7.2
Median	2.5
Mode	---
Standard Deviation	19.7
Variance	389.6
Skewness	15.55
Kurtosis	513.74
Coeff. of Variability	2.73
Range Minimum	0.0
Range Maximum	1,496.2
Range Width	1,496.2
Mean Std. Error	0.04



Forecast: 1.2 % ct shampoo (cont'd) **Cell: B18**

Percentiles:

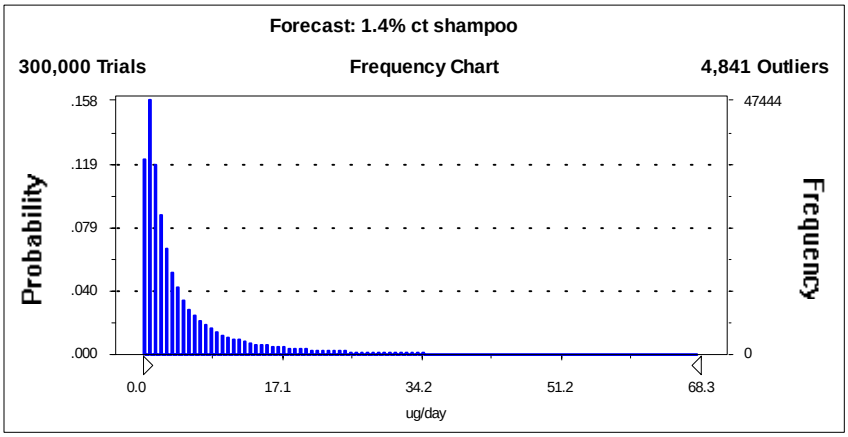
Percentile	ug/day
0%	0.0
5%	0.3
10%	0.5
15%	0.7
20%	0.9
25%	1.0
30%	1.3
35%	1.5
40%	1.8
45%	2.1
50%	2.5
55%	2.9
60%	3.5
65%	4.2
70%	5.1
75%	6.3
80%	8.1
85%	10.8
90%	15.6
95%	27.1
100%	1,496.2

End of Forecast

Forecast: 1.4% ct shampoo **Cell: B19**

Summary:
Display Range is from 0.0 to 68.3 ug/day
Entire Range is from 0.0 to 1,745.6 ug/day
After 300,000 Trials, the Std. Error of the Mean is 0.0

Statistics:	Value
Trials	300000
Mean	8.4
Median	2.9
Mode	---
Standard Deviation	23.0
Variance	530.3
Skewness	15.55
Kurtosis	513.74
Coeff. of Variability	2.73
Range Minimum	0.0
Range Maximum	1,745.6
Range Width	1,745.6
Mean Std. Error	0.04



Forecast: 1.4% ct shampoo (cont'd) **Cell: B19**

Percentiles:

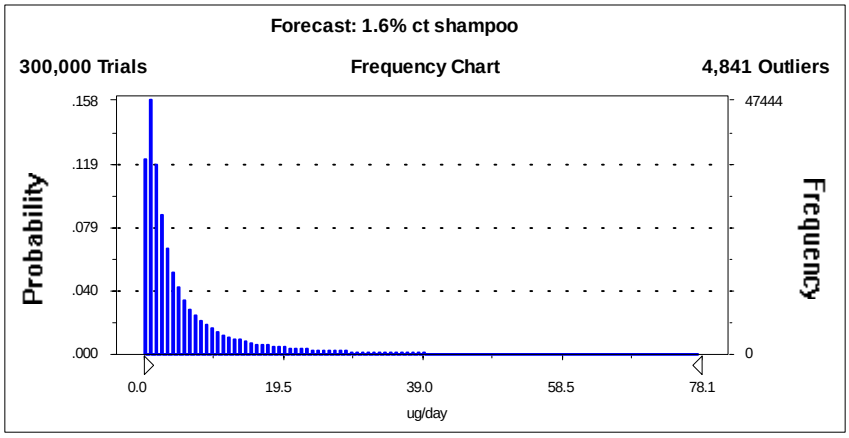
Percentile	ug/day
0%	0.0
5%	0.4
10%	0.6
15%	0.8
20%	1.0
25%	1.2
30%	1.5
35%	1.7
40%	2.1
45%	2.4
50%	2.9
55%	3.4
60%	4.0
65%	4.9
70%	6.0
75%	7.4
80%	9.4
85%	12.5
90%	18.2
95%	31.7
100%	1,745.6

End of Forecast

Forecast: 1.6% ct shampoo **Cell: B20**

Summary:
Display Range is from 0.0 to 78.1 ug/day
Entire Range is from 0.0 to 1,994.9 ug/day
After 300,000 Trials, the Std. Error of the Mean is 0.0

Statistics:	Value
Trials	300000
Mean	9.6
Median	3.3
Mode	---
Standard Deviation	26.3
Variance	692.6
Skewness	15.55
Kurtosis	513.74
Coeff. of Variability	2.73
Range Minimum	0.0
Range Maximum	1,994.9
Range Width	1,994.9
Mean Std. Error	0.05



Forecast: 1.6% ct shampoo (cont'd) **Cell: B20**

Percentiles:

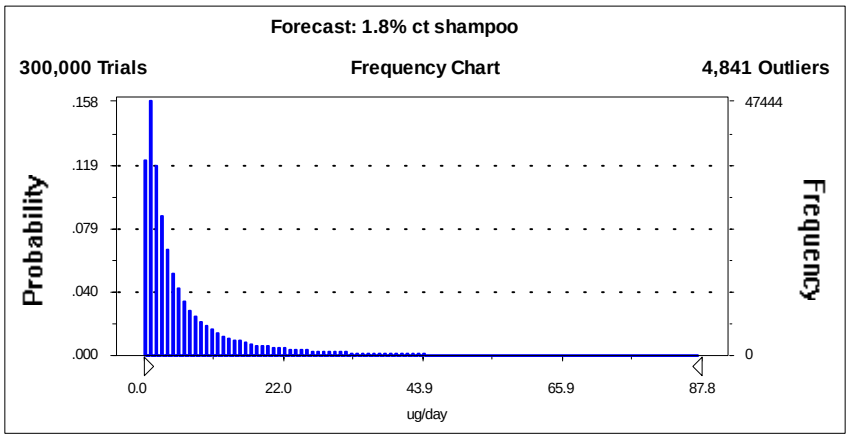
Percentile	ug/day
0%	0.0
5%	0.5
10%	0.7
15%	0.9
20%	1.1
25%	1.4
30%	1.7
35%	2.0
40%	2.4
45%	2.8
50%	3.3
55%	3.9
60%	4.6
65%	5.6
70%	6.8
75%	8.5
80%	10.8
85%	14.3
90%	20.8
95%	36.2
100%	1,994.9

End of Forecast

Forecast: 1.8% ct shampoo **Cell: B21**

Summary:
Display Range is from 0.0 to 87.8 ug/day
Entire Range is from 0.0 to 2,244.3 ug/day
After 300,000 Trials, the Std. Error of the Mean is 0.1

Statistics:	Value
Trials	300000
Mean	10.8
Median	3.7
Mode	---
Standard Deviation	29.6
Variance	876.6
Skewness	15.55
Kurtosis	513.74
Coeff. of Variability	2.73
Range Minimum	0.0
Range Maximum	2,244.3
Range Width	2,244.3
Mean Std. Error	0.05



Forecast: 1.8% ct shampoo (cont'd) **Cell: B21**

Percentiles:

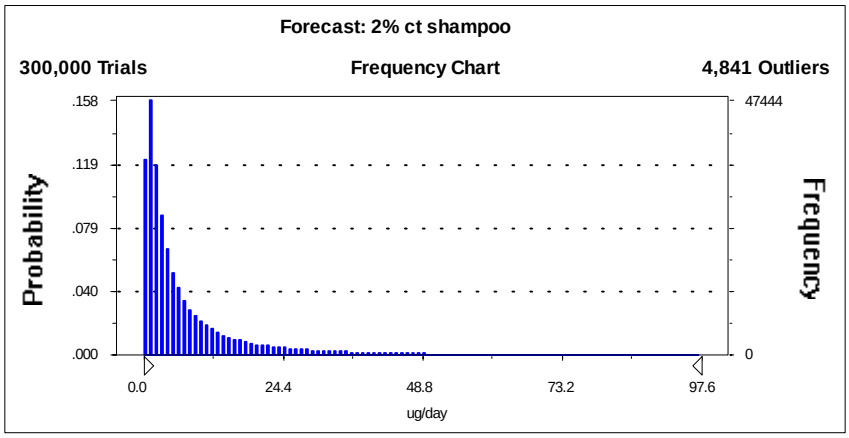
Percentile	ug/day
0%	0.0
5%	0.5
10%	0.8
15%	1.0
20%	1.3
25%	1.6
30%	1.9
35%	2.2
40%	2.6
45%	3.1
50%	3.7
55%	4.4
60%	5.2
65%	6.3
70%	7.7
75%	9.5
80%	12.1
85%	16.1
90%	23.4
95%	40.7
100%	2,244.3

End of Forecast

Forecast: 2% ct shampoo **Cell: B22**

Summary:
Display Range is from 0.0 to 97.6 ug/day
Entire Range is from 0.0 to 2,493.7 ug/day
After 300,000 Trials, the Std. Error of the Mean is 0.1

Statistics:	Value
Trials	300000
Mean	12.0
Median	4.1
Mode	---
Standard Deviation	32.9
Variance	1,082.2
Skewness	15.55
Kurtosis	513.74
Coeff. of Variability	2.73
Range Minimum	0.0
Range Maximum	2,493.7
Range Width	2,493.7
Mean Std. Error	0.06



Forecast: 2% ct shampoo (cont'd) **Cell: B22**

Percentiles:

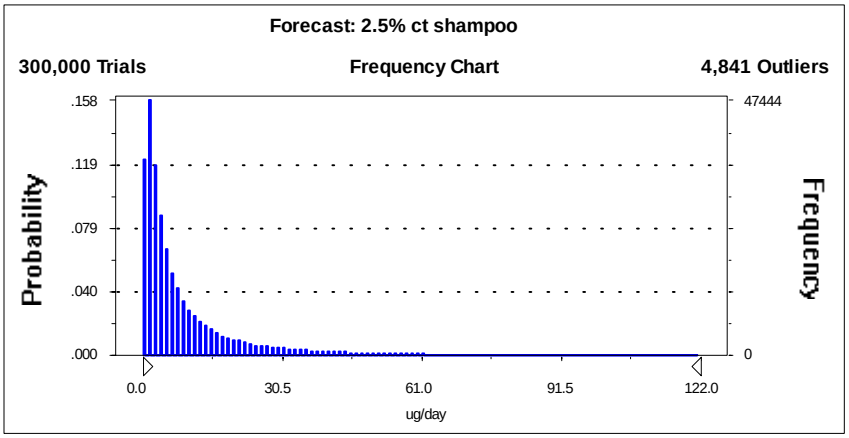
Percentile	ug/day
0%	0.0
5%	0.6
10%	0.9
15%	1.1
20%	1.4
25%	1.7
30%	2.1
35%	2.5
40%	2.9
45%	3.5
50%	4.1
55%	4.9
60%	5.8
65%	7.0
70%	8.5
75%	10.6
80%	13.4
85%	17.9
90%	26.0
95%	45.2
100%	2,493.7

End of Forecast

Forecast: 2.5% ct shampoo **Cell: B23**

Summary:
Display Range is from 0.0 to 122.0 ug/day
Entire Range is from 0.0 to 3,117.1 ug/day
After 300,000 Trials, the Std. Error of the Mean is 0.1

Statistics:	Value
Trials	300000
Mean	15.1
Median	5.1
Mode	---
Standard Deviation	41.1
Variance	1,691.0
Skewness	15.55
Kurtosis	513.74
Coeff. of Variability	2.73
Range Minimum	0.0
Range Maximum	3,117.1
Range Width	3,117.1
Mean Std. Error	0.08



Forecast: 2.5% ct shampoo (cont'd) **Cell: B23**

Percentiles:

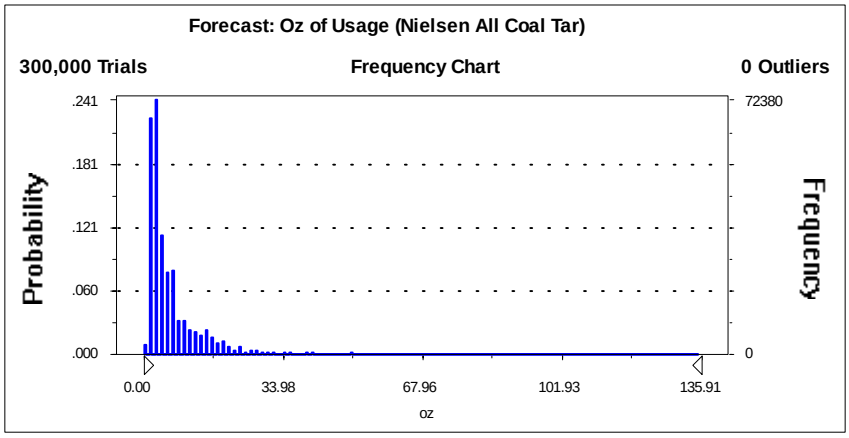
Percentile	ug/day
0%	0.0
5%	0.7
10%	1.1
15%	1.4
20%	1.8
25%	2.2
30%	2.6
35%	3.1
40%	3.7
45%	4.3
50%	5.1
55%	6.1
60%	7.2
65%	8.7
70%	10.6
75%	13.2
80%	16.8
85%	22.4
90%	32.4
95%	56.6
100%	3,117.1

End of Forecast

Forecast: Oz of Usage (Nielsen All Coal Tar) **Cell: B12**

Summary:
Display Range is from 0.00 to 135.91 oz
Entire Range is from 0.00 to 135.91 oz
After 300,000 Trials, the Std. Error of the Mean is 0.02

Statistics:	Value
Trials	300000
Mean	7.42
Median	4.20
Mode	---
Standard Deviation	8.80
Variance	77.37
Skewness	4.82
Kurtosis	45.20
Coeff. of Variability	1.18
Range Minimum	0.00
Range Maximum	135.91
Range Width	135.91
Mean Std. Error	0.02



Forecast: Oz of Usage (Nielsen All Coal Tar) (cont'd) **Cell: B12**

Percentiles:

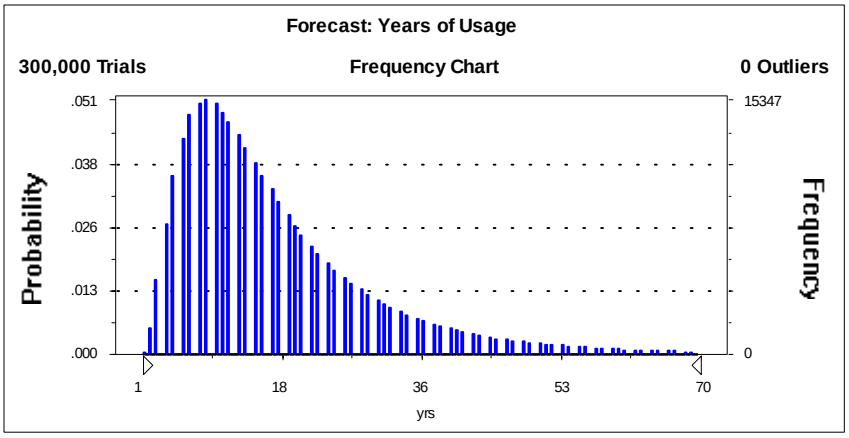
Percentile	oz
0%	0.00
5%	1.78
10%	2.07
15%	2.26
20%	2.45
25%	2.87
30%	3.21
35%	3.49
40%	3.72
45%	3.95
50%	4.20
55%	4.44
60%	5.58
65%	6.29
70%	7.59
75%	8.19
80%	10.22
85%	12.88
90%	16.20
95%	22.34
100%	135.91

End of Forecast

Forecast: Years of Usage **Cell: B11**

Summary:
Display Range is from 1 to 70 yrs
Entire Range is from 1 to 70 yrs
After 300,000 Trials, the Std. Error of the Mean is 0

Statistics:	Value
Trials	300000
Mean	18
Median	14
Mode	9
Standard Deviation	12
Variance	142
Skewness	1.48
Kurtosis	5.37
Coeff. of Variability	0.68
Range Minimum	1
Range Maximum	70
Range Width	69
Mean Std. Error	0.02



Forecast: Years of Usage (cont'd) **Cell: B11**

Percentiles:

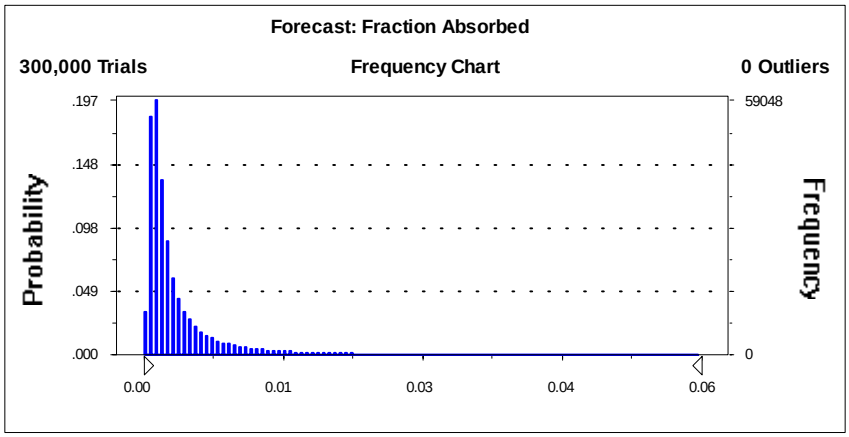
Percentile	yrs
0%	1
5%	5
10%	6
15%	7
20%	8
25%	9
30%	10
35%	11
40%	12
45%	13
50%	14
55%	16
60%	17
65%	19
70%	20
75%	23
80%	25
85%	29
90%	34
95%	42
100%	70

End of Forecast

Forecast: Fraction Absorbed **Cell: B10**

Summary:
Display Range is from 0.00 to 0.06
Entire Range is from 0.00 to 0.06
After 300,000 Trials, the Std. Error of the Mean is 0.00

Statistics:	Value
Trials	300000
Mean	0.0042
Median	0.0024
Mode	---
Standard Deviation	0.0051
Variance	0.0000
Skewness	3.4899
Kurtosis	18.6806
Coeff. of Variability	1.2285
Range Minimum	0.0003
Range Maximum	0.0568
Range Width	0.0565
Mean Std. Error	0.0000



Forecast: Fraction Absorbed (cont'd) **Cell: B10**

Percentiles:

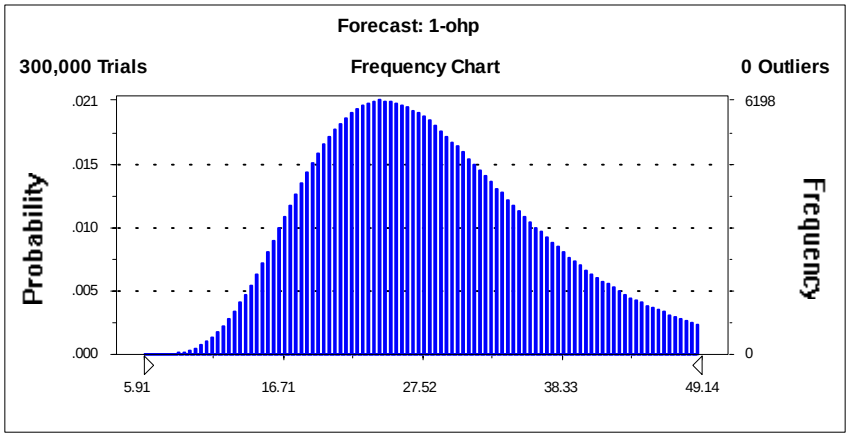
Percentile	Value
0%	0.0003
5%	0.0010
10%	0.0011
15%	0.0013
20%	0.0014
25%	0.0015
30%	0.0017
35%	0.0018
40%	0.0020
45%	0.0022
50%	0.0024
55%	0.0026
60%	0.0029
65%	0.0033
70%	0.0037
75%	0.0044
80%	0.0053
85%	0.0067
90%	0.0092
95%	0.0144
100%	0.0568

End of Forecast

Forecast: 1-ohp **Cell: B4**

Summary:
Display Range is from 5.91 to 49.14
Entire Range is from 5.91 to 49.14
After 300,000 Trials, the Std. Error of the Mean is 0.02

Statistics:	Value
Trials	300000
Mean	27.86
Median	26.99
Mode	---
Standard Deviation	8.24
Variance	67.87
Skewness	0.38
Kurtosis	2.55
Coeff. of Variability	0.30
Range Minimum	5.91
Range Maximum	49.14
Range Width	43.23
Mean Std. Error	0.02



Forecast: 1-ohp (cont'd) **Cell: B4**

Percentiles:

Percentile	Value
0%	5.91
5%	15.70
10%	17.72
15%	19.22
20%	20.50
25%	21.66
30%	22.75
35%	23.81
40%	24.86
45%	25.92
50%	26.99
55%	28.10
60%	29.27
65%	30.51
70%	31.87
75%	33.36
80%	35.07
85%	37.10
90%	39.63
95%	43.13
100%	49.14

End of Forecast

APPENDIX E

Consideration of Mouse Data in the Derivation of a NSRL

E.1 Introduction

In the absence of suitable epidemiological data, data collected from animal bioassays can be used to estimate the carcinogenic potency of a chemical. If multiple bioassays have been conducted in more than one species and/or strain, then the species that is most biologically comparable to humans is preferred, but if that is not known, then the most sensitive endpoint in the most sensitive species is usually selected and used to estimate potency. For a variety of reasons, including differences in pharmacokinetics and the mode of action for a particular chemical, the animal model employed in bioassays may not accurately predict the potency of a chemical in humans. Depending on the factors involved, potency estimated from animal data may potentially overestimate or underestimate the corresponding human potency of a chemical by several orders of magnitude.

As discussed in the main report, there is a wealth of evidence that coal tars produce skin tumors in mice when tested in skin painting assays. Nevertheless, while useful as a qualitative screen for carcinogenic potential, a quantitative human health risk assessment based on the mouse tumor data is not appropriate. The available data suggest that the mouse is not a suitable animal model for estimating potential risks of skin cancer in the human following dermal exposure to coal tars because of the pharmacokinetic and pharmacodynamic differences between mouse and human skin (Sections 2.3 and 2.4).

In addition, no dermal bioassays have been conducted in the mouse with pharmaceutical grade coal tar that have the necessary quantitative information for dose-response analysis. As discussed in Section 2.1, the only available chronic bioassay conducted in the mouse for which the necessary data are available for dose-response analysis is a study conducted by the Fraunhoffer Institute (1997). However, this study involved dermal application of creosote, rather than pharmaceutical grade coal tar. The available data indicate that the mixture of PAHs, not just one component, is critical to the carcinogenic potency of coal tar mixture (Section 2.1). Because the composition of a coal tar mixture is critical to carcinogenic potency, it would be inappropriate to attempt to use the data for one mixture, such as creosote, to estimate the carcinogenic potency of another mixture, such as pharmaceutical grade coal tar.

Although the mouse is not a suitable model, this appendix contains the results of an attempt to quantify the potential carcinogenic risks in humans from use of coal tar-containing shampoo using the only available bioassay in the mouse, the Fraunhoffer Institute (1997) study, for which data are suitable for dose-response analysis. This analysis also incorporates the available quantitative data on the pharmacokinetic species differences that would impact the potential carcinogenicity of PAHs in the skin of each species. However, due to the vast uncertainty in the relevance of the results in the mouse to the human, it must be stressed that the results of this analysis should be used with caution. The available data are not adequate to quantitatively characterize the differences in pharmacodynamics between mouse and human skin that may impact the carcinogenic potential of PAH mixtures.

The first step in conducting a dose-response analysis using mouse data is to estimate the human equivalent doses (HEDs) based on the doses applied to the animals in the bioassay (Fraunhoffer 1997). This step incorporates the available quantitative information on the pharmacokinetic differences between mouse and human skin. These HEDs are then used in combination with the response data from the animal bioassay for dose-response modeling. The following sections describe this process, as well as the data considered. An initial analysis is conducted with a NSRL expressed as a single value; however, for direct comparison to the NSRL estimated based on epidemiological studies conducted in psoriasis patients (Pittelkow et al. 1981), a NSRL expressed as absorbed dose is also presented in an uncertainty analysis that characterizes some of the potential uncertainty in the estimation of a NSRL using mouse data. Further discussion of the uncertainties in using the mouse skin data is also provided.

E.2 Estimation of the Human Equivalent Dose

When a NSRL is derived using the mouse data, the average daily doses applied in the animal bioassay should be converted to a HED for incorporation into a dose-response model (USEPA 1996, 1999). Estimation of the HED involves incorporating the quantitative information, when available, on the differences in pharmacokinetics, as well as pharmacodynamics, between the experimental species (mouse) and the target species (human). In the case of systemic toxicity from dermal exposure, species differences in the pharmacokinetics of a chemical in passing through the skin compartment should be

considered; however, when the target tissue is the skin, the amount of chemical in the skin compartment should be considered. Initial movement into the skin is determined by absorption; therefore, species differences in absorption should be considered. In the case of PAHs, the carcinogenic moiety is thought to be a metabolite(s) of the parent compound(s) that is formed via metabolism in the skin compartment. Differences in capacity of the metabolic pathway that is the rate-limiting pathway (aryl hydrocarbon hydroxylase [AHH]) in the formation of the active metabolite(s) should, therefore, be considered. Finally, species differences in removal or clearance from the skin compartment should be incorporated in the estimation of the HED. The result of these pharmacokinetic adjustments is an applied dose in the human that would result in the same target tissue dose in the skin as that estimated in the animal resulting from application of the bioassay doses.

An additional consideration is the species differences in pharmacodynamics, or response, from exposure of the target tissue to the carcinogenic moiety. No quantitative information is available specifically for PAHs, although available qualitative information suggests that the promotional pathway operative in the development of skin tumors resulting from PAHs exposure in the mouse may not be operative in humans (see Section 2.4). At the least, an adjustment for differences in response between species should include a comparison of the fraction of the target organ (skin) that is exposed to the chemical of interest, in this case coal tar. It has sometimes been assumed that response would scale by total surface area exposed, which would suggest a much larger sensitivity to skin carcinogens in larger species. This assumption, however, would be inconsistent with the empirical evidence for cross-species scaling of cancer, which demonstrates similar cancer responses for whole organs receiving pharmacokinetically equivalent exposures (USEPA 1992). That is, empirical evidence for cancer of systemic tissues (e.g., Crump et al. 1989) is consistent with a similar lifetime risk of cancer for exposures of the entire tissue or organ at pharmacokinetically equivalent lifetime average daily doses (LADDs) (USEPA 1992), and not consistent with the seemingly logical assumption that cancer risk should be proportional to the number of cells at risk (and hence tissue volume). The suggestion to scale skin cancer equivalence by applied surface area is analogous to the latter assumption, with skin depth

assumed equal across species. Using whole-organ pharmacodynamic equivalence, based on USEPA (1992), leads to the adjustment for fraction of organ (skin) exposed.

The derivation of HEDs for a dermal application, incorporating both pharmacokinetic and pharmacodynamic species differences, is described in the following equation. The adjustment for the pharmacokinetic differences is shown in brackets, and consists of multiple factors that include the difference in absorption between the mouse and the human, the difference in AHH activity in each species, and the expected difference in clearance from the skin compartment. A subscript of *A* denotes an animal value, while a subscript of *H* denotes a human value.

$$HED(mg/day) = AA * \left[\frac{\frac{FA_A}{FA_H} \times \frac{AHH_A}{AHH_H} \times CLR}{\frac{SV_A}{SV_H}} \right] * \frac{PAF_A}{PAF_H}$$

where:

AA	=	Average daily applied amount in the animal dose group of the bioassay (µg/day)
FA	=	Fraction of applied dose that is absorbed (unitless)
AHH	=	AHH activity (pmol/min/mg protein)
CLR	=	Ratio of the body weight-adjusted clearance from the skin compartment (unitless)
SV	=	Volume of the skin at application site (cm^3)
PAF	=	Fraction of total body surface area to which coal tar is applied (unitless)

The initial step in conducting a dose-response analysis using the mouse data is to estimate the HEDs for each bioassay average daily applied dose using this equation. These HEDs are then incorporated into the dose-response analysis, rather than the applied average daily doses in the animal bioassay. The model can then predict the HED that is associated with a 1×10^{-5} risk, which is the human NSRL in µg/day. The basis for the parameters used to derive HEDs using the average daily amount applied from the Fraunhoffer (1997) study (0, 0.1, 0.3, 1, 3, or 9 mg for 2 days/week) is described in the following sections.

Average Daily Applied Amount (AA)

The animal data reported by Warshawsky et al. (1993) and Fraunhofer (1997) provide clear evidence that the potential carcinogenicity of coal tar mixtures is entirely dependent on the constituents in the mixture and cannot be estimated based on BaP content alone. Further, the results of Grimmer et al. (1984) and NIOSH (1979) indicate that make-up of the PAH fraction is responsible for activity. Therefore, for the average daily applied amount, the doses of coal tar applied in the bioassay were used for the dose-response analysis adjusted to an average daily amount.

Fraction of Applied Dose That is Absorbed (FA)

The estimation of the fraction of applied dose that is absorbed in the mouse is based on results from a study conducted by Storm et al. (1990). Because no information on absorption of coal tar was available in the mouse, absorption information on BaP was used as a surrogate for absorption in the bioassay. In the study conducted by Storm et al. (1990), the absorption of BaP was determined *in vitro* in the intact skin of female SENCAR or BALB/c mice. The skin samples were placed in flow though diffusion cells and allowed to equilibrate for 1 hour before application of ^{14}C-radiolabelled BaP (3 µg/cm^2 in 10 µl acetone). The receptor fluid was collected in 6-hour fractions over a 24-hour period and analyzed for parent compound and metabolites. At 24 hours, very little radiolabel was observed in the receptor fluid (less than 5% of the administered dose). Approximately 60% of the applied dose was absorbed by the skin and remained in the skin over the 24-hour period. Therefore, the fraction of applied dose that is absorbed following application of coal tar was assumed to be 0.6 in the mouse.

In the human, information on the fraction of applied amount absorbed relevant for the exposure of concern is provided in a shampooing study reported by Van Schooten et al. (1994). In this study, 11 volunteers washed their hair with a total of 20 grams of coal-tar shampoo. Shampooing was conducted for two, 30-second durations. Based on the total urinary excretion of 1-hydroxypyrene (1-OH-P), it is estimated that approximately 0.5% of the applied dose was absorbed (see Section 4.1). Therefore, the fraction of applied dose that is absorbed in the human following the use of coal tar shampoo would be approximately 0.005.

AHH Activity (AHH)

Species differences in AHH activity are critical in estimating a NSRL for coal tar shampoo, because these enzymes are thought to be the rate-limiting step in the metabolism of PAHs in the skin to the active carcinogenic moiety. Several studies have been conducted in which the basal and induced activity of AHH following application of individual or mixtures of PAHs have been evaluated in the mouse or the human (Bickers and Kappas 1978; Bickers et al. 1984; Das et al. 1986; Kinoshita and Gelboin 1972; Levin et al. 1972; Storm et al. 1990; Thompson and Slaga 1976). The results from these studies indicate that the average basal activity in the mouse is approximately 1.5 pmol/min/mg protein, while the average basal activity in the human is approximately 0.2 pmol/min/mg protein. Information on inducibility of this enzyme also indicates greater induction in the mouse. Maximal induced AHH activity for the studies combined was approximately 11.5 pmol/min/mg protein in the mouse and 0.7 pmol/min/mg protein in the human.

For the estimation of the HED, it is assumed that the exposures to coal tar in both the bioassay in the animals and from shampooing resulted in maximally induced AHH. This assumed relationship results in a factor of approximately 16.5 greater activity in the mouse, compared to the human (11.5 pmol/min/mg protein ÷ 0.7 pmol/min/mg protein). However, data in humans indicate that exposures resulting from shampooing may not result in maximal induction of AHH (Hukkelhoven et al. 1984). In this study, one to ten applications (0.5 ml) of a commercial coal tar preparation (diluted or undiluted) were applied to a 3 cm^2 section of the scalp of human volunteers. Each application was applied at 12-hour intervals. During this 12-hour interval, the volunteers were asked not to wash their hair. At the end of each 12-hour period, the hair was washed to remove excess coal tar and hair follicles were plucked and evaluated for AHH activity. Basal measurements were determined based on hair follicles plucked from the side of the scalp opposite the application site. With the undiluted coal preparation, maximal induction was reached after three applications; however, with the diluted coal tar preparation, maximal induced activity was not achieved after ten applications. Therefore, there is uncertainty in the ratio of the AHH activity in the animal at the bioassay doses compared to the AHH activity in the human following application of coal tar shampoo. If AHH in the human is not maximally induced

following application of coal tar shampoo, then the ratio of AHH activity between the animal and human would increase, possibly as great as a factor of 4, if no induction occurred in the human.

Clearance Ratio (CLR)

There are differences in both the skin volume and the blood perfusion rate to the skin compartment that would influence the rate of clearance of chemicals from the mouse skin versus the human skin, if the primary clearance mechanism was perfusion (Brown et al. 1997; Davies and Morris 1993). However, available information on the clearance of PAHs from the skin would suggest clearance is diffusion-limited. Very little BaP was cleared from the skin compartment of either the mouse (Storm et al. 1990) or the human (Storm et al. 1990; Wester et al. 1990) following application for 24 hours. Although these studies also indicate differences in clearance from the skin compartment between mice and humans, no experimental data on the rate of clearance of PAHs from the skin compartment in either the mouse or the human were located. In the absence of specific data, allometric scaling can be used to account for differences in clearance rates in different species (USEPA 1992).

Results from allometric studies have indicated that rates, such as clearance, tend to maintain proportionality with body weight to the 3/4 power (Adolph 1949; Schmidt-Nielsen 1970, 1975, 1984; Travis 1990). Scaling in this manner results in a clearance rate that is smaller per unit weight or volume in larger animals. Therefore, for clearance of PAHs from the skin, the relationship between mice and humans was assumed to scale by $BW^{3/4}$. The resulting adjustment factor for clearance is approximately 0.15, which assumes that the mouse clears PAHs from the skin at about 7 times the rate of clearance in the human skin.

Volume of the Skin (SV)

Adjusting the applied amount by the volume of the skin available for absorption for a dermal application is similar to adjusting doses on a body weight basis (mg/kg body weight/day) when conducting a dose-response assessment for an oral exposure. In an oral application, adjusting the dose on a per kilogram body weight basis assumes a uniform distribution within the body of either the

experimental or target species, resulting in the estimate of an initial average concentration following exposure. Adjustment of the dose by the default body weight to the 3/4 power adjustment for animal-to-human scaling corrects the estimated dose for the species to species differences in clearance (as discussed previously), resulting in an estimate of the average concentration over time, or the area under the concentration curve, in the target species. Therefore, adjusting by the volume of the skin at the application site assumes a uniform distribution within the skin compartment, resulting in an estimate of the initial average concentration of PAHs in the skin compartment.

The volume of the target area of the skin was estimated by combining the surface area to which the coal tar was applied with the depth or thickness of the skin in that area. In the mouse bioassay, the coal tar was applied to an area that was 10% of the total body surface area (Fraunhoffer 1997) or approximately 3.6 cm^2, assuming an average body surface area of 36 cm^2 in the mouse (Harkness and Wagner 1983). Thickness of mouse skin is approximately 0.003 cm (Klein-Szanto et al. 1991). Using these values, the volume of the exposed mouse skin was estimated to be approximately 0.01 cm^3.

For the human, the relevant surface area exposed during a shampooing event was assumed to be the scalp and the hands. If shampooing occurs in the shower, some exposure may occur to other parts of the body, but would be negligible compared to the exposure to the hands and the scalp (FDA, OTC 160369). The area of the scalp is approximately 700 cm^2 based on a study reported by Kalopissis (1986) and the area of the hands is approximately 1000 cm^2 (USEPA 1997). The depth of the skin for the scalp and back of the hands is approximately 0.01 cm (Klein-Szanto et al. 1991), while the depth of the skin for the palms is approximately 0.06 cm (ICRP 1975). Using these values in the human, an area-weighted volume of skin exposed to coal tar shampoo was estimated of approximately 41 cm^3.

Fraction of Total Body Surface Area (PAF)

As reported (Fraunhoffer 1997), application of the coal tar compounds was to 10% of the total surface area of the mouse. Therefore, the fraction of total body surface area exposed in the mouse was assumed to be 0.1.

The total surface area of the human is approximately 20,000 cm^2. If the surface area exposed is the area of the scalp and hands, then the total area exposed is approximately 1,700 cm^2. The resulting fraction of total body surface area exposed in the human is then approximately 9% or 0.09. As discussed in detail in Section 4.0, the total residue remaining after shampooing and rinsing either at the sink or in the shower was the same and the residue was mainly found on the scalp and hands.

E.3 Derivation of the NSRL Expressed in Applied Amount Using Fraunhoffer (1997)

The HEDs in µg/day were used in place of the applied amount (µg/day) in the mouse in combination with the response data in the mouse reported by Fraunhoffer (1997) for the dose-response assessment. Because of the decreased survival in the high-dose group, less than lifetime exposure (78 weeks versus 104 weeks), and the availability of individual animal data, a time-to-tumor analysis was conducted using the Multistage Weibull model (TOX_RISK® 2000). Use of a time-to-tumor model can adjust for competing risks for the development of a tumor, such as early deaths, while quantal models do not have this capability. It also has the capability of extrapolating risks beyond the duration of the study. Therefore, a NSRL could be estimated based on the expected risk of skin cancer in the mouse if the study had been conducted for the default lifespan (104 weeks). The form of the model used was

$$P(d,t) = 1 - e^{\left[-\left(q_0 + q_1 d + \cdots + q_k d^k \right) \cdot \left(t - t_0 \right)^c \right]}$$

$$\text{for } c \geq 1,\ t_0 \geq 0,\ q_i \geq 0,\ i = 0, \ldots, k \text{ and}$$

$$k = \#\text{ of dose groups} - 1$$

The resulting output from the dose-response modeling was a potency estimate in $(\mu g/day)^{-1}$ in humans and estimate of the lower bound on dose associated with 1×10^{-5} risk, or the NSRL in µg/day in terms of applied dose or absorbed dose. The resulting NSRL estimated using the mouse data reported by Fraunhofer (1997) is 11,300 µg/day in terms of applied dose or 56.5 µg/day in terms of absorbed dose.

E.4 Estimation of the Uncertainty in the Mouse NSRL

To address how imprecise the mouse estimate could be and to include some uncertainty bounds around the mouse estimate, a Monte Carlo analysis was conducted to develop a distribution of NSRLs based on the mouse data. To achieve this, distributions were developed for each parameter involved in the estimation of a HED for each of the mouse bioassay doses in the equation provided in Section E.2., with the exception of the applied amount and the pharmacodynamic adjustment factor (*PAF*) as in the following equation:

$$HED(mg/day) = AA * \left[\frac{\frac{FA_A}{} \times \frac{AHH_A}{AHH_H} \times CLR}{\frac{SV_A}{SV_H}} \right]$$

where:

AA	=	Average daily applied amount in the animal dose group of the bioassay (µg/day)
FA	=	Fraction of applied dose that is absorbed (unitless)
AHH	=	AHH activity (pmol/min/mg protein)
CLR	=	Ratio of the body weight-adjusted clearance from the skin compartment (unitless)
SV	=	Volume of the skin at application site (cm^3)

With the currently available data, one cannot bound the uncertainty in the pharmacodynamics between species, and a distribution would provide a perception of accuracy greater than is appropriate given the data. The value selected for *PAF* (the fraction of total body surface to which coal tar is applied) represents a worst-case assumption that human skin has the same susceptibility as mouse skin to the carcinogenicity of coal tar constituents, an assumption which is contradicted by the mechanistic and xenografts data discussed in Section 2.4.

In the dose-response analysis discussed in the previous section, a Multistage Weibull time-to-tumor model was used to account for the much shorter duration of exposure in the 9 mg/day dose group and for the less than lifetime exposure (78 weeks) for the remaining dose groups. However,

because this analysis is intended to give an idea of the potential uncertainty in the estimate and because the Multistage Weibull model could not be readily used in this type of analysis, the high-dose group was removed from the analysis and the Multistage model used with a crude time-to-tumor adjustment [(duration of study/average lifespan)^3]. To insure that this analysis did not result in a NSRL estimate vastly different from the NSRL discussed above (11,300 µg coal tar/day), the HEDs and tumor incidence used in the original analysis for all of the dose groups (excluding the 9 mg/day group) were used in a dose-response analysis using the Multistage model with the crude time-to-tumor adjustment. The resulting NSRL (10,500 µg coal tar/day) was slightly more conservative, but consistent with the NSRL estimated using the Multistage Weibull model.

For comparison to the NSRL estimated based on Pittelkow et al (1981), which is expressed as absorbed dose, a NSRL based on the mouse data that was expressed as absorbed dose was needed. In order to provide a "ball park" for comparison, the adjustment for fraction of applied dose that is absorbed in the human (FA_H) was removed from consideration in the above equation, resulting in HEDs expressed as "absorbed dose."

As stated previously, for the Monte Carlo analysis, distributions for each of the parameters described in Section E.2 were needed. Because this is an exercise to provide a "ball park" comparison to the NSRL based on Pittelkow et al. (1981) and to provide a crude estimation of the potential uncertainty in the mouse estimate, the data for the derivation of the distributions were limited to the data relied upon for the estimation of the NSRL based on applied amount. The basis for the distributions used is provided in the following sections.

Fraction of Applied Dose That is Absorbed (FA)

The estimation of the fraction of applied dose that is absorbed in the mouse was estimated to be approximately 0.6 based on the data provided by Storm et al. (1990). The data presented by Storm et al. (1990) were in graphical form, with bars representing the standard error on the mean (SEM) provided. These data were digitized to obtain a mean of 0.63 and a SEM of 0.082. This information was used to develop a distribution of fraction of applied dose that is absorbed in the mouse. The

distribution was assumed to be truncated normal, with the truncation based on other available data including Sanders et al. (1986). This information was not included in the point estimate of the fraction of absorbed dose because of uncertainties associated with the results of the study. In Sanders et al. (1986), BaP was applied *in vivo* to the skin of mice and absorption estimated based on radioactivity data in the urine/feces. However, there were significant recovery problems in the study, increasing the uncertainty in the authors' estimates of absorbed dose. However, the definition of the distribution for the uncertainty analysis attempted to include both the qualitative and quantitative information on absorption in the mouse, to insure that the distribution would encompass any available data.

AHH Activity (AHH)

The available quantitative information AHH activity in the mouse and human is provided in Table E-1. As discussed previously, for the estimation of the HED it is assumed that the exposures to coal tar in both the bioassay in the animals and from shampooing resulted in maximally induced AHH. The available information indicates that for the mouse the average induced AHH activity is 11.5 pmol/min/mg protein, which is the point estimate considered in the estimation of the NSRL based on applied amount. The range of values for induced AHH activity in the mouse spans from 8.33 to 16.43 pmol/min/mg protein (Das et al. 1986; Kinoshita and Gelboin 1972; Thompson and Slaga 1976). This information was used to define a triangular distribution for AHH activity in the mouse, with a mean of 11.5 pmol/min/mg protein and a minimum and maximum of 8.33 and 16.43 pmol/min/mg protein, respectively.

A similar distribution was used for the human induced AHH activity, based on the data provided in Table E-1. For the human, a mean of 0.7 pmol/min/mg protein was estimated based on all of the available information, with a minimum of 0.165 pmol/min/mg protein and a maximum of 2.29 pmol/min/mg protein (Alvares et al. 1972; Bickers and Kappas 1978; Bickers et al. 1984; Das et al. 1986; Levin et al. 1972). This information was used to define a triangular distribution for AHH activity in the human.

Clearance Ratio (CLR)

No experimental data were located on the rate of clearance of PAHs from the skin compartment in either the mouse or the human. In the absence of specific data, allometric scaling was used based on the results from allometric studies that indicate the rates, such as clearance, tend to maintain proportionality with body weight to the 3/4 power (Adolph 1949; Schmidt-Nielsen 1970, 1975, 1984; Travis 1990). For clearance of PAHs from the skin, the relationship between mice and humans was assumed to scale by $BW^{3/4}$. The resulting adjustment factor for clearance is approximately 0.15, which assumes that the mouse clears PAHs from the skin at about 7 times the rate of clearance in the human skin. To develop a possible minimum value for a distribution, it could be assumed that clearance of both the parent compound and the metabolites would scale by $BW^{3/4}$, resulting in a factor of $(BW^{3/4})^2$ or $BW^{9/16}$. The resulting minimum adjustment factor for clearance would be 0.034. The maximum adjustment factor for clearance was assumed to scale by BW, resulting in a factor of 1. The resulting triangular distribution was assumed to range from 0.034 (minimum) to 1.0 (maximum), with the mostly likely value being 0.15.

Volume of the Skin (SV)

The volume of the target area of the skin was estimated by combining the surface area to which the coal tar was applied with the depth or thickness of the skin in that area. As discussed previously, in the mouse bioassay (Fraunhoffer 1997) coal tar was applied to an area that was 10% of the total body surface area (Fraunhoffer 1997) or approximately 3.6 cm^2. No information was available on the variation on the experimental surface area of application. However, it was assumed that the application site could vary by approximately ± 2%. There was also no information on variation in the thickness of mouse skin, which is approximately 0.003 cm (Klein-Szanto et al. 1991); therefore, it was assumed that the thickness of mouse skin could vary by approximately ±10%. Based on these assumptions, a triangular distribution was developed for surface area of mouse skin exposed to PAHs during the Fraunhoffer (1997) bioassay. The resulting minimum value was 0.0095 cm^3, with a maximum value of 0.0121 cm^3 and a most likely or mean value of 0.0108 cm^3.

For the human, the mean surface area exposed during a shampooing event was assumed to be the scalp and the hands, and was estimated based on data on the surface area of the scalp and hands (Kalopissis 1986; USEPA 1997) and depth of the skin for the scalp and hands (ICRP 1975; Klein-Szanto et al. 1991). No information was available on the variation in the surface area and depth of the skin of the scalp; however, this information was available for the hands, as well as the skin in general in USEPA (1997) and ICRP (1975). As with the mouse, a triangular distribution of surface area of the skin of the exposed was developed for a shampooing exposure scenario. The most likely or mean value, as discussed in Section E.2, was 41 cm^3, with a minimum value of 30.75 cm^3 and a maximum value of 51.25 cm^3, based on the available information on the variation in the depth of human skin and in the surface area of the hands.

Results of the Monte Carlo Analysis

For each iteration in the Monte Carlo analysis, each of the above distributions was sampled to develop one set of HEDs associated with the animal bioassay doses reported in Fraunhoffer (1997) (0.1, 0.3, 1, and 3 mg/day). The HEDs and incidence data for skin tumors from Fraunhoffer (1997) were then incorporated into TOX_RISK for Windows and the Multistage model fit to the data to estimate the lower bound on dose associated with a one in a 100,000 risk. This process was repeated for 100,000 iterations, resulting in the development of a distribution of NSRLs expressed as absorbed dose.

The resulting distribution of NSRLs based on the mouse data, and expressed as absorbed dose, had a median or 50th percentile of 58.5, with a 95th percentile of 300 and a 5th percentile of approximately 9. Therefore, it appears that the “ball park” NSRL based on the mouse data could vary by at least a factor of approximately ±6. However, this range of estimates is based on the worst-case assumption (*PAF=1*) that the mouse and human are equally susceptible to the carcinogenicity of coal tar constituents. The mechanistic and xenografts data discussed in Section 2.4 clearly indicate that the human is much less susceptible, if at all, to these effects. Therefore, this range represents the lower bound on an uncertainty distribution for the NSRL that has infinity as the upper bound.

E.5 Qualitative Uncertainties in the Estimation of a NSRL Based on Mouse Data

There are numerous uncertainties in using the mouse skin tumor data for coal tars in an assessment to determine the risk of the development of these tumor types in humans following application of coal tar shampoo. The main uncertainty is the relevance of using skin tumor incidence data in mouse skin to estimate risk of skin cancer in humans. As discussed in Sections 2.3 and 2.4, there are clear differences in the pharmacokinetic capabilities of mouse and human skin. Moreover, the response of human skin to transformation and tumor promotion indicates that human skin is less sensitive than mouse skin to tumor induction in response to PAHs (Section 2.4). More importantly, the basis for that difference may be explained by the modes by which tumor promotion in response to PAHs are thought to occur in mouse and human skin (Section 2.4).

Carcinogenic constituents in coal tar have both initiating and promoting properties. In the context of a multistage process for the production of skin tumors, initiation alone, in the absence of promotion, either self-promotion as would be expected by a complete carcinogen, such as BaP, or promotion by other agents present in coal tar, will not result in tumor production. In mouse skin, the mode of tumor promotion for several PAHs has been demonstrated to be by the same mode as that for the classic tumor promotor, TPA. The evidence strongly indicates that tumor promotion in mouse keratinocytes may be prostaglandin-dependent, while tumor promotion in human keratinocytes is not (Section 2.4). This suggests that the "trigger" for activation of the tumor promotional cascade is different in mouse and human skin, and that the "trigger" required for PAH induction of skin tumors in the mouse is not operative in human skin. Consequently, the observations seen in mouse skin are of questionable relevance as a quantitative predictor of human skin tumor risk. This raises the question as to whether the mouse skin tumor model is an acceptable model for assessing the risk of skin tumors in humans.

If the animal data are to be used in a dose-response analysis for the estimation of the risk of skin cancer in the human, pharmacokinetic and pharmacodynamic differences should be considered to "scale" from the animal to the human (USEPA 1992). This indicates another major uncertainty in using mouse data for the estimation of human risk of skin cancer, and that is the selection of the relevant

target tissue dose metric for animal-to-human extrapolation. Ideally, the dose metric would be the amount of the active metabolite(s) for each of the PAHs that is available to the target cell in the skin. However, this cannot be quantified with existing kinetic data. The dose metric used in this analysis is the concentration of the total metabolites formed via the AHH pathway, based on the available information on the activity of AHH in both mouse and human skin, that remains in the target organ, the skin. This dose metric was selected because it is the rate-limiting step in the formation of the active metabolite(s). Comparative differences in absorption, metabolism clearance, skin volume, and fraction of surface area applied were used to adjust the animal applied amount and estimate HEDs.

Other dose metrics have been suggested for use in animal-to-human extrapolation for dermal exposures, e.g., mg/cm^2 or mg/kg/day; however, these dose metrics do not consider the key pharmacokinetic differences between mouse and human skin, and, consequently, are poor estimators for species extrapolation. Moreover, use of other dose metrics results in estimates of NSRLs that vary by orders of magnitude and are inconsistent with the epidemiological data for patients treated for years with coal tar-containing ointments. For example, assuming that species scaling is accomplished based on $body\ weight^{2/3}$ results in a NSRL of 2 µg/day, while scaling based on the mass in milligrams applied to mouse skin results in a NSRL of 0.02 µg/day, both of which are assumed to be a lower bound on dose at a one in 100,000 risk. However, if these estimated NSRLs are compared with known applications for psoriasis treatments, the NSRLs appear overly conservative. For example, patients undergoing Goeckerman therapy for psoriasis or other skin conditions are exposed to huge amounts of coal tar, ranging up to lifetime average applied amounts of 36,000 µg/day, based on information reported in Pittelkow et al. (1981). If either of these two dose metrics was an accurate reflection of the potential risk to humans, then it would follow that every patient treated for psoriasis with coal tar should develop skin cancer. After more than 80 years of clinical use, such a dramatic increase in skin cancer has not been reported.

Because some quantitative information on the pharmacokinetic and pharmacodynamic differences between the mouse and human skin is available, an attempt was made to incorporate this quantitative information in the development of a NSRL based on the mouse data. As discussed

previously, pharmacokinetic and pharmcaodynamic adjustments were used with the applied amount in the mouse to estimate HEDs for use in the dose-response analysis. The methods used for adjusting the applied amount in the mouse for the estimation of a HED incorporated methods that are similar for estimating HEDs for systemic effects. In addition to the inherent uncertainties in using mouse skin tumor data to estimate human risk of skin cancer, there are also uncertainties associated with the animal-to-human correction factors used in the estimation of the HEDs for the dose-response analysis.

In deriving HEDs for use in the dose-response analysis, it was assumed that maximal induction of AHH would occur in both the mouse and the human following the relevant exposures to coal tar. This is more likely with the animal following application of the bioassay doses that are in milligrams, but not the human following application to the scalp, based on data reported by Hukkelhoven et al. (1984). Results from this study indicate that following ten applications of diluted coal tar, maximal induction was not achieved in volunteers. If human AHH activity is induced to some degree following a shampooing event, the human activity could be any point between the basal average and the maximal induced activity. Depending on the level of induction in the human, the ratio of the animal AHH activity to the human could vary from a factor of approximately 16, used in the derivation of the HEDs, up to a factor 68.

The percent of dermal absorption in the mouse used in the estimation of the HED was based on *in vitro* data reported by Storm et al. (1990). In this study, radiolabeled BaP was applied to intact mouse skin in a flowthrough system and amount of radiolabel in the skin and the receptor fluid measured over a 24-hour period. There is some uncertainty as to whether these results would be representative of the dermal absorption that would be expected following application of a coal tar mixture as in the bioassay reported by Fraunhoffer (1997). Based on information reported by Dankovic et al. (1989), BaP absorption may be decreased if applied in a coal tar mixture. In this study, BaP alone in a volatile organic solvent or BaP in a PAH mixture was applied to the shaved skin of female mice, and dermal half-lives of disappearance of the PAHs from the surface of the skin over a 24-hour period was determined. The estimated biological half-lives indicate disappearance from the skin of BaP in a volatile organic solvent at a faster rate (half-life = 3.0 hours) than BaP in a PAH

mixture (half-life = 6.7 hours). This indicates that absorption of BaP may be slower from a mixture, and possibly less based on decreased levels of metabolites observed if BaP was in a mixture. However, this method does not account for any volatilization of BaP from the application site. The study provides no data that could be used to quantify the potential absorption of BaP only or BaP in the mixture. However, the results from this study provide qualitative evidence of possible uncertainties in using an absorption fraction based on BaP only as representative of a mixture of PAHs.

For scaling animal-to-human clearance from the skin compartment, we are assuming that most likely clearance from the skin compartment would scale on a body weight$^{3/4}$. This is a very conservative assumption that is based on overall clearance of chemicals from the body and generally applies to rates such as cardiac output, minute volume, glomerular filtration rate, etc. It assumes that clearance from the skin of the mouse occurs at a seven-fold faster rate than in the human. Although the mouse may clear PAHs from the skin compartment more readily than humans, this is probably related more to thickness of the skin, because clearance from the skin is a diffusion-limited process. Mouse skin is thinner than human skin, and, therefore, chemicals may diffuse through the mouse skin at a faster rate, but probably less than seven-fold faster.

Some uncertainties exist in the estimation of the volume of skin exposed in both the mouse and the human. The surface area of the site of application in the mouse reported by Fraunhoffer (1997) is vague, with the authors reporting that the test material was applied to approximately 10% of the surface area of the animals. However, the variation in the application site was not reported, as well as the average total body surface area for the mice used in the study. The only total body surface area documented in the available literature was a value for a mouse whose body weight was less than the average body weight of the mice used in the Fraunhoffer (1997) study. Therefore, the estimated volume of skin exposed in the mouse may be underestimated, depending on the actual surface area associated with the application site. However, the volume of skin exposed in humans may also be underestimated. The surface area considered in the estimation of the volume of skin exposed was limited to the scalp and one side of the hands. If shampooing occurred during a shower, the surface area could be underestimated. Some residue, although slight, could remain on parts of the body other

than the hands or the scalp. Volume in both species could also be slightly underestimated because of lateral movement within the skin, although such lateral movement is generally minimal.

In adjusting for the potential pharmacodyanamic differences between mouse skin and human skin in the estimation of a HED, the fraction of surface area to which the coal tar compound applied was used as the appropriate measure. It is normally assumed that response would scale by surface area for the skin, not fraction of area applied. For this assessment, it was assumed as with systemic organs, that the risk of skin cancer scales from animal to human with equivalent dose to the entire organ, not to the number of cells at risk. This is an area of uncertainty, because it assumes that what is known about response for systemic organs would apply in the case of the skin. However, the NSRL currently estimated using the mouse skin tumor data already appears overly conservative when compared to the doses applied for psoriasis treatment (Pittelkow et al. 1981). If it was assumed that the risk of skin cancer would scale from animal to human based on total number of cells at risk, or surface area applied, the result would be a 500-fold decrease in the NSRL estimated based on the Fraunhoffer (1997) study. Again, this value is inconsistent with the epidemiological data, and all patients treated for psoriasis with coal tar ointment would be expected to develop skin cancer.

Overall, the available data on the differences, both pharmacokinetically and pharmacodynamically, between mouse skin and human skin question the use of mouse skin tumor data in the estimation of human skin cancer risk. Because of the numerous uncertainties that are inherent in using the mouse skin tumor data, use of the epidemiological study should be preferred.

Table E-1
Aryl Hydrocarbon Hydroxylase Inducibility in Mice and Human Skin

Mouse (pmole/min/mg protein)		Human (pmole/min/mg protein)		Reference
Basal	Induced	Basal	Induced	
Induced with Benz(a)anthracene				
1.1 1.3 1.7	8.5 12.5 12.5			Thompson and Slaga (1976) (1) Thompson and Slaga (1976) (1) Kinoshita and Gelboin (1972) (1)
		0.1 0.1 0.06	0.4 0.33 0.165	Levin et al. (1972 (2) Alvares et al. (1972) (2) Bickers et al. (1984) (2)
Induced with DMBA				
1.2	8.33			Kinoshita and Gelboin (1972) (1)
Induced with Coal Tar				
0.59	16.43	0.45 0.045	2.29 0.165	Das et al. (1986) (3) Bickers and Kappas (1978) (4)
Uninduced				
3.25		0.24		Storm et al. (1990) (5)

1 BA (100-200 µmoles) applied to mouse skin 8-24 hours before harvesting; BaP (100 nmoles) added to epidermal cell homogenate for 30 minutes. Control mice vehicle only.
2 Human neonatal foreskin or adult epidermal homogenates incubated with or without BA (10-13 µmoles) for 16-24 hours; BaP (100 nmoles) added for 30 minutes.
3 Crude coal tar (0.1 ml) was applied to a human skin xenograft and to distal host mouse skin for 24 hours before harvesting epidermis; BaP (100 nmoles) added for 30 minutes.
4 A 20% coal tar solution was applied to the skin of adult human volunteers for 24 hours under occluded dressing. Biopsy whole skin homogenates from treated and untreated areas incubated with BaP (100 nmoles) for 30 minutes.
5 Application of 3 µg BaP/cm^2 applied for 30 minutes.

References

Adolph EF. 1949. Quantitative relations in the physiological constitution of mammals. Science 109:579-585.

Alvares AP, Kappas A, Levin W, Conney AH. 1972. Inducibility of benzo(a)pyrene hydroxylase in human skin by polycyclic hydrocarbons. Clin Pharmacol Ther 14:30-40.

Bickers DR, Kappas A. 1978. Human skin aryl hydrocarbon hydroxylase. Induction by coal tar. J Clin Invest 68:1061-1068.

Bickers DR, Mukhtar H, Dutta-Choudhury T, Marcelo CL, Voorhees JJ. 1984. Aryl hydrocarbon hydroxylase, epoxide hydrolase, and benzo(a)pyrene metabolism in human epidermis: comparative studies in normal subjects and patients with psoriasis. J Invest Dermatol 83:51-56.

Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Beliles RP. 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health 13:407-484.

Crump K, Allen B, Shipp A. 1989. Choice of dose measure for extrapolating carcinogenic risk from animals to humans: an empirical investigation of 23 chemicals. Health Phys 57(Suppl 1):387-393.

Dankovic DA, Wright CW, Zangar RC, Springer DL. 1989. Complex mixture effects on the dermal absorption of benzo(a)pyrene and other polycyclic aromatic hydrocarbons from mouse skin. J Appl Toxicol 9:239-244.

Das M, Asokan P, Don P, Krueger GG, Bickers DR, Mukhtar H. 1986. Carcinogen metabolism in human skin grafted onto athymic nude mice: a model system for the study of human skin carcinogenesis. Biochem Biophys Res Commun 138:33-39.

Davies B, Morris T. 1993. Physiological parameters in laboratory animals and humans. Pharmaceu Res 10:1093-1095.

Fraunhofer Institute of Toxicology. 1997. Dermal Carcinogenicity Study of Two Coal Tar Products (CTP) by Chronic Epicutaneous Application in Male CD-1 Mice (78 Weeks). Final Report. Fraunhofer Institute of Toxicology, Federal Republic of Germany. October 1997.

Grimmer G, Brune H, Deutsch-Wenzel R, Dettbarn G, Misfeld J, Abel U, Timm J. 1984. The contribution of polycyclic aromatic hydrocarbons to the carcinogenic impact of emission condensate from coal-fired residential furnaces evaluated by topical application to the skin of mice. Cancer Lett 23:167-176.

Harkness JE, Wagner JE, eds. 1983 The Biology and Medicine of Rabbits and Rodents. 2nd ed. Lea & Febiger, Philadelphia.

Hukkelhoven M, Vromans L, van Pelt F, Keulers R, Vermorken A. 1984. In vivo induction of aryl hydrocarbon hydroxylase in human scalp hair follicles by topical application of a commercial coal tar preparation. Cancer Lett 23:135-143.

International Commission on Radiological Protection (ICRP). 1975. Report of the Task Group on Reference Man. Vol 23. Pergamon Press, New York.

Kalopissis G. 1986. Toxicology and hair dyes. In: Zviak C, ed. The Science of Hair Care. Marcel Dekker, New York. pp. 287-308.

Kinoshita N, Gelboin H. 1972. The role of aryl hydrocarbon hydroxylase in 7,12-dimethylbenz(a)anthracene skin tumorigenesis: on the mechanism of 7,8-benzoflavone inhibition of tumorigenesis. Cancer Res 32:1329-1339.

Klein-Szanto AJP, Conti CJ, Aldaz CM. Skin and oral mucosa. 1991. In: Haschek MW, Rousseauc CG, eds. Handbook of Toxicologic Pathology. Academic Press, Inc., New York. pp. 165-193.

Levin W, Conney A, Alvares A, et al. 1972. Induction of benzo[a]pyrene hydroxylase in human skin. Science 176:419-420.

NIOSH. 1979. Tumors Inducted in C3H/HeJ Mice by Coal Tar Neutral Subfractions. U.S. Department of Health Education and Welfare. November 1979.

Pittelkow M, Perry H, Muller S, Maughan W, O'Brien P. 1981. Skin cancer in patients with psoriasis treated with coal tar. Arch Dermatol 117:465-468.

Sanders CL, Skinner C, Gelman RA. 1986. Percutaneous absorption of 7,10^{14}C-benzo(a)pyrene and 7,12^{14}C-dimethylbenz(a)anthracene in mice. J Environ Pathol Toxicol Oncol 7:25-34.

Schmidt-Nielsen K. 1970. Energy metabolism, body size, and problems of scaling. Fed Proc 29:1524-1532 (As cited in USEPA 1992).

Schmidt-Nielsen K. 1975. Scaling in biology: the consequences of size. J Exp Zool 194:287-308 (As cited in USEPA 1992).

Schmidt-Nielsen K. 1984. Scaling: Why is Animal Size so Important? Cambridge University Press, Cambridge (As cited in USEPA 1992).

Storm J, Collier S, Stewart R, Bronaugh R. 1990. Metabolism of xenobiotics during percutaneous penetration: Role of absorption rate and cutaneous enzyme activity. Fundam Appl Toxicol 15:132-141.

Thompson S, Slaga TJ. 1976. Mouse epidermal aryl hydrocarbon hydroxylase. J Invest Dermatol 66:108-111.

TOX_RISK®. 2000. Toxicology Risk Assessment Program. Version 5.0. Developed by the K.S. Crump Group, Inc. under contract to Electric Power Research Institute, Palo Alto, CA.

Travis CC. 1990. Tissue dosimetry for reactive metabolites. Risk Anal 10:317-321.

USEPA. 1992. A cross-species scaling factor for carcinogen risk assessment based on equivalence of $mg/kg^{3/4}/day$; notice. Draft report. Fed Regist 57(109):24152-24173.

USEPA. 1996. Proposed Guidelines for Carcinogen Risk Assessment. EPA/600/P-92/003C. Office of Research and Development, Washington, DC. April, 1996.

USEPA. 1997. An Approach for Calculating Risk from Dermal Exposure to Benzo(a)pyrene Based on Point of Contact Effects. Draft.

USEPA. 1999. Science Advisory Board. Review of the USEPA 1996 Proposed Guidelines for Carcinogen Risk Assessment.

Van Schooten F-J, Moonen EJC, Rheinsburger E, van Agen B, Thijssen HHW, Kleinjans JCS. 1994. Dermal uptake of polycyclic aromatic hydrocarbons after hairwash with coal-tar. Lancet 344:1505-1506.

Warshawsky D, Barkley W, Bingham E. 1993. Factors affecting carcinogenic potential of mixtures. Fundam Appl Toxicol 20:376-382.

Wester RC, Maibach HI, Bucks DAW, Sedik L, Melendres J, Liao C, DiZio S. 1990. Percutaneous absorption of [^{14}C]DDT and [^{14}C]benzo(a)pyrene from soil. Fundam Appl Toxicol 15:510-516.