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RE: NTP Methyleugenol Studies

In compliance with the National Toxicology Program's (NTP) mission to keep our colleagues informed of the current NTP findings during on-going studies, a copy of the Pathology Working Group (PWG) report and the Summary Pathology Tables for the chronic gavage studies on Methyleugenol (CAS No. 93-15-2) are enclosed for your review.

The NTP assembles a Pathology Working Group to review every study and to resolve any differences between the study laboratory and quality assessment pathology evaluations. Please note that the PWG conclusion of the study results is based solely on the pathology for this study and may not reflect final NTP conclusions. In determining final conclusions, the NTP assesses a broad array of information that includes other results from this study and historical control data.

All study data are subject to an NTP retrospective audit and the interpretation may be modified based on the findings. The technical report for this study is currently being prepared for presentation to the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee with an anticipated peer-review date of October 1998.

The Summary Pathology Tables along with a wide variety of NTP information are also available in electronic format on the world-wide web. For example, the NTP Annual Plan, abstracts of NTP Reports, study data, and the status of all NTP studies may be viewed electronically with access to the internet and a Web browser such as Netscape Navigator or Internet Explorer.

To locate the NTP home page, use the URL <http://ntp-server.niehs.nih.gov/>. The NTP individual animal and summary pathology tables, survival tables, and survival and growth curves may be accessed from the NTP homepage selecting "Testing Information and Study Results" and then choosing "Data of Completed Studies." Comments on the usefulness of this site and suggestions for improvement are encouraged.

Please contact Central Data Management (CDM) at (919)541-3419 if you have any questions. You may also fax your requests for information to CDM at (919)541-3687 or send them via e-mail to cdm@niehs.nih.gov.



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Hard copies of documents such as NTP Technical Reports, short-term Toxicity Reports, and the Report on Carcinogens are available from the Environmental Health Information Service (EHIS). You can contact EHIS by phone at (919) 541-3841, by fax at (919)541-0273, or by e-mail at ehis@niehs.nih.gov.

Sincerely,



William Eastin, Ph.D.
Head, Information Systems & Central Files
Environmental Toxicology Program

Encls: Rats & Mice, PWG, Pathology Summary Tables
cc: Dr. J. Bucher
Dr. K Abdo
Central Data Management

National Toxicology Program Methyleugenol Studies.

Methyleugenol is a natural constituent of a large number of essential oils including rose, basil, hyacinth, pimento, citronella, anise (400 ppm), nutmeg, mace, and cinnamon and laurel fruits and leaves. The chemical has also been identified in bananas, and black pepper.

Methyleugenol was given GRAS (generally recognized as safe) status in 1965 and is approved by the FDA for use in food (21CFR 121.1164). It is used as a flavoring agent in jellies (52 ppm), baked goods (13 ppm), nonalcoholic beverages (10 ppm), chewing gum, candy (11 ppm), pudding, relish and ice cream (4.8 ppm). The council of Europe has listed the acceptable daily intake as 5 mg/kg/day. It is also used as a fragrance in perfumes 0.3%-0.8%, creams and lotions (0.01%-0.05%), and soaps and detergents (0.02%-0.2%). One of the major uses for methyleugenol is as an insect attractant. It was used in California in 1982, in combination with malathion, to control an outbreak of oriental fruit flies.

Toxicity and carcinogenicity studies were conducted in rats and mice by administering the chemical by gavage once daily, five days per week for up to 2 years. Additionally a stop exposure study was conducted in rats to determine whether the rodents can recover from the effects of the chemical. Toxicokinetic and metabolism studies in rats and mice as well as *in vitro* DNA adduct formation studies using rat and human liver slices were also conducted.

Serum from volunteers consuming ginger snaps will be analyzed to determine the bioavailability of this agent in people who consume foods containing methyleugenol. The information obtained from this study will be used for evaluating the health risk to humans from normal exposure to this chemical based on comparisons with blood levels in rodents from the NTP toxicology studies.

The NTP assembles a Pathology Working Group (PWG) to review every study and to resolve any differences between the study laboratory and quality assessment pathology evaluations. It should be noted that in determining final conclusions, the NTP assesses a broad array of information that takes into account all data obtained from the NTP evaluation of each agent and that of the historical controls plus relevant published literature. In addition, all study data are subject to an NTP retrospective audit and the interpretation may be modified based on these findings.

However, in its mandate to keep the public informed in a timely manner of current National Toxicology Program (NTP) study results, summary pathology tables are made available for distribution by mail and are also posted on the NTP website (http://ntp-server.niehs.nih.gov/Main_Pages/NTP_TR_Index.html) as the PWG reviews are completed. All of the study data for methyleugenol are currently under evaluation and a technical report is being prepared for presentation to the NTP Board of Scientific Counselors' Peer Review Subcommittee on October 30, 1998.



CHAIRPERSON'S REPORT
PATHOLOGY WORKING GROUP REVIEW
METHYLEUGENOL (C60991B)
27- AND 52-WEEK INTERIM SACRIFICE
AND 2-YEAR CHRONIC ORAL GAVAGE
STUDY IN F344 RATS CONDUCTED AT
BATTELLE-COLUMBUS

Date of Pathology Working Group Review: May 12 and 14, 1998

Participants: James Hailey, D.V.M.; NIEHS
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(PWG Chairperson)

STUDY DESIGN

Male and female F344 rats were exposed to Methyleugenol by a single oral gavage dose, excluding weekends and Battelle holidays, at levels of 0, 37, 75, 150, or 300 mg/kg/day for up to 104 weeks, except for the 300 mg/kg group which was given the vehicle only at the end of the 12th month of dosing (Day 361) until termination (Week 104). The control animals received 0.5 aqueous methylcellulose, the vehicle for Methyleugenol. Five animals/sex were sacrificed at the 27-



week and 52-week interim periods in the 0 and 300 mg/kg groups only. Table 1 summarizes the animal disposition for the study

TABLE 1
Male Rats

Dose (mg/kg)	0	37	75	150	300
Animals in Study	60	50	50	50	60
Moribund Sacrifice	15	18	15	20	23
Natural Deaths	15	15	17	29	26
Interim Sacrifice	10	0	0	0	10
Terminal Sacrifice	20	16	15	0	0
Other	0	1	3	1	1

Female Rats

Dose (mg/kg)	0	37	75	150	300
Animals in Study	60	50	50	50	60
Moribund Sacrifice	17	16	14	26	25
Natural Deaths	11	9	13	12	9
Interim Sacrifice	10	0	0	0	10
Terminal Sacrifice	22	25	22	11	16
Other	0	0	1	1	0

SUMMARY

Administration of Methyleugenol by gavage, under the conditions of this study, was associated with the following histopathologic lesions:



1. The presence and dose-related increased incidence of neuroendocrine cell hyperplasia, neuroendocrine tumors and malignant neuroendocrine tumors of the glandular stomach in male and female rats. Atrophy, involving glandular epithelial cells, was also present in exposed male and female rats in the glandular stomach.
2. The presence and dose-related increased incidence of a spectrum of non-neoplastic and proliferative lesions within the livers of treated male and female rats. Non-neoplastic lesions consisted of focal cystic degeneration; hepatocellular hypertrophy; oval cell hyperplasia; and bile duct hyperplasia (females), atypical hyperplasia and cysts. Proliferative lesions included increased numbers of eosinophilic foci; hepatocellular adenomas and carcinomas; hepatocholangiomas and hepatocholangiocarcinomas; cholangiomas and cholangiocarcinomas.
3. An apparent dose-related increased incidence of malignant mesothelioma in male animals. Mesothelioma was present on either the epididymis, testis or peritoneum or combinations thereof.
4. An increased severity of renal nephropathy in the 150 and 300 mg/kg males and 300 mg/kg females. In the males, an increased combined incidence of renal tubule hyperplasia and adenoma was noted in the 75, 150, and 300 mg/kg exposed groups.
5. A slight increased incidence of benign pheochromocytomas of the adrenal medulla in high-dose female rats.
6. A slight increased incidence of mammary gland fibroadenomas in the 75 and 150 mg/kg male rats.
7. An increased number of subcutaneous fibromas/fibrosarcomas in the exposed male rats. The increased incidence was not dose-dependent.
8. An increased incidence of bone marrow hyperplasia (hypercellularity) particularly in the treated female animals.



9. The presence of cytologic alteration (decreased eosinophilic granularity) in the submandibular salivary gland of nearly every exposed male and female animal.

Evaluation of the strength and significance of the pathology findings must await generation of the final tables.

CONDUCT OF THE PATHOLOGY WORKING GROUP

Prior to the PWG, the chairperson reviewed the pathology tables, the original study pathologist's (SP) narrative, the quality assessment (QA) report prepared by the quality assessment pathologist (QAP) and microscopic slides of potential target organs and selected lesions with discrepancies in diagnoses between the SP and QAP.

The following potential target organs and/or specific organ diagnoses were reviewed by the QAP from all control and treated rats as directed by NTP (letter dated April 7, 1993).

Liver:

1. Review all non-neoplastic discrepancies between the SP and QAP.
2. Review all neoplasms (because of good agreement between SP and QAP only discrepancies may be elected to be reviewed).

Glandular Stomach:

1. Review all lesions.



Epididymis, Testes and Peritoneum:

1. Review all lesions.

Mammary Gland and Skin:

1. Review all lesions.

Uterus:

1. Review all lesions.

Kidney and Organs With Lesions Related to Nephropathy:

1. Review all non-neoplastic discrepancies between the SP and QAP.
2. Review all neoplasms.
3. Review only the discrepancies in organs with lesions related to nephropathy (e.g. mineralization and fibrous osteodystrophy).
4. Review all lesions of the parathyroid gland.

Salivary Gland:

1. Review all discrepancies between the SP and QAP.

Bone Marrow:

1. Review all lesions.
2. Determine the appropriateness of QAP's approach to diagnosis of hyperplastic lesions.

Spleen:

1. Review all non-neoplastic discrepancies between the SP and QAP.
2. Review all neoplasms (because of good agreement between the SP and QAP only discrepancies may be elected to be reviewed).



Adrenal Medulla:

1. Review all lesions.

Additionally, most tumor diagnoses in organs not previously mentioned above were reviewed from all animals in all groups.

The PWG Chairperson selected a set of 203 slides for review by the PWG. These slides included representative examples of potential treatment-related lesions, lesions for which there was a difference of opinion between the SP and QAP and lesions selected because of general interest (see Chairperson's PWG Worksheets). All slides were examined by each participant without knowledge of the dose group or diagnoses rendered by the SP and QAP. Final diagnoses for the lesions presented were determined by the consensus of the PWG participants.

On May 12, only the treatment-related glandular stomach lesions were discussed by the PWG. Methyleugenol-induced stomach lesions were described in both the mice and rats with a series of 2 x 2 Kodachromes which illustrated the spectrum of lesions observed. Prior to examining the PWG slides the appropriate diagnostic nomenclature for the stomach lesions was discussed and agreed upon by the members of the PWG.



PWG RESULTS

Glandular Stomach

Treatment-related non-neoplastic and neoplastic lesions were diagnosed by the SP in the glandular portion of the stomach. Grossly, these lesions were described as either a diffuse thickening or, more commonly, as nodule(s)/mass(es). The non-neoplastic lesions diagnosed by the SP included epithelial atrophy, epithelial hyperplasia (focal, diffuse) and atypical epithelial hyperplasia. The neoplastic lesions included adenoma and carcinoma of the glandular epithelium. Atypical hyperplasia according to the SP "was characterized by highly disorganized and microscopically invasive epithelial growth within the hyperplast. gastric lesions". The SP even considered these lesions as potential "carcinoma in situ" lesions although their malignant potential was unclear. The stomach neoplasms, according to the SP, varied in appearance. Most were solid with fewer acinus forming tumors. The SP thought that most tumors originated from the parietal cell or, less frequently, from the chief or zymogenic cells. None of the neoplasms were believed by the SP to originate from mucous-producing cells or represent gastric carcinoids.



Following the QA review, the QAP confirmed the presence of epithelial atrophy and atypical hyperplasia. However, many of the SP's reported cases of hyperplasia were diagnosed as atypical hyperplasia by the QAP. In addition, the QAP diagnosed many more cases of carcinoma in the methyleugenol exposed animals. Almost all of the SP's adenomas were considered to be carcinomas by the QAP. Based upon the site of proliferation and general appearance, the QAP believed that some of these lesions represented gastric carcinoids or hyperplasias originating from the endocrine cells or enterochromaffin-like (ECL) cells found along the basilar portion of the fundic glands.

As mentioned previously, the PWG discussed the apparent origin of the lesions in question and appropriate diagnostic terminology before examining the PWG slides containing the discrepancies. Drs. Gordon Hard and Spencer Streett supported the ECL origin of these lesions and further remarked that these lesions were typical of what was seen in



their reported studies. In keeping with current terminology suggested by the STP/ARP/AFIP: Guides for Toxicologic Pathology (Prantz JD, Betton G, Cartwright NE, Crissman JW, Macklin AW and Maronpot RR (1991) Proliferative lesions of the non-glandular and glandular stomach in rats, GI-3.), the diagnostic terms neuroendocrine cell hyperplasia (severity); neuroendocrine tumor and neuroendocrine tumor, malignant were agreed upon by the PWG.

Following the PWG review of the stomach slides, the PWG confirmed the spectrum of methyleugenol-induced non-neoplastic and neoplastic gastric lesions. Female rats were more severely affected than the males. In addition, the PWG eliminated "epithelium" as a further site designation for all lesions of the glandular stomach.

Atrophy was characterized by the thinning of the fundic mucosa due to a loss of the parietal and chief epithelial cells. This lesion, as noted in the early time points, began near the limiting ridge (margo plicans). With time, this lesion became more diffuse along the fundus. Atrophy was observed in both sexes and was evident in the 300 mg/kg male and female animals by the 27-week interim sacrifice period. By the end of the study, the majority of exposed animals had



atrophy. The mechanism(s) involving the loss of parietal and/or chief cells did not appear to be due to overt necrosis or apoptosis since these types of lesions were not readily apparent.

Neuroendocrine cell hyperplasia represented a proliferation of ECL cells that tended to be focal and did not markedly expand the mucosa or greatly distort the adjacent fundic glands. ECL cells appeared to be round to polyhedral, faintly basophilic or amorphilic with round to oval nuclei and usually a single nuclei. They often appeared in nests or clusters and could be seen extending up or down within the mucosa. Sevier-Munger silver stains demonstrated their argyrophilic positivity.

Neuroendocrine tumor and neuroendocrine tumor, malignant represented a "biologic continuum" from hyperplasia. The distinction between marked hyperplasia and neuroendocrine tumor was somewhat arbitrary. In general, these "benign" tumors were also confined to the mucosal and were non-invasive. However, in contrast to hyperplasia, neuroendocrine tumors were more diffuse, or nodular resulting in expansion of the mucosa and distortion of the adjacent



glands. Within neuroendocrine tumors, the neoplastic cells appeared more pleomorphic but retained many of their ECL cell features including a strong positivity with Sevier-Munger staining.

Malignant neuroendocrine tumors are usually diagnosed by their large size, increased cell atypia and invasion through the muscularis mucosa. The latter being the most consistent hallmark of malignant progression. Malignant tumors were characterized by either diffuse mucosal-involvement and/or nodular appearance. Most were solid tumors consisting of either pleomorphic ECL cells or mixtures of ECL cells and glandular (zymogenic) cells. Marked cellular anaplasia or a high mitotic rate were not characteristic of most neoplasms. In the more malignant neoplasms, different cell patterns were common suggesting differentiation from one or more cell types of the glandular epithelium (neuroendocrine cell, chief cells, parietal cells), or possibly divergent differentiation of a stem-cell population. It has also been postulated that ECL tumor cells may produce growth factors (paracrine hormones) that stimulate glandular epithelial cells to proliferate and become dysplastic. Sevier-Munger staining in these tumors was variable. In general, malignant cells with faint, foamy cytoplasm were often silver-positive while less foamy cells



or glandular-appearing cells were silver-negative. Metastases were occasionally noted with the liver, lungs and abdominal lymph nodes affected.

The development of methyleugenol-induced proliferative neoplastic lesions in the fundic mucosa may be related by a secondary mechanism to loss of parietal cells (atrophy) with subsequent decrease in gastric acidity (achlorohydrria) and hypergastrinemia. Hypergastrinemia is known to have a trophic effect on the fundic mucosa, including ECL hypertrophy, hyperplasia and the development of tumors of neuroendocrin origin (carcinoids). This form of carcinogenesis usually demonstrates a dose-threshold phenomenon. In the treated animals from this study, neuroendocrine neoplasms were observed as low as the 75 mg/kg group in females and the 150 mg/kg group in males. Neuroendocrine cell hyperplasia was present in the 37 mg/kg females.

Several squamous cell neoplasms (papilloma and squamous cell carcinoma) were also confirmed by the PWG in exposed animals. These exophytic growths were essentially present on the limiting ridge. The biological significance of these neoplasms was not clear but because of their low overall incidence and lack of any appreciable dose-response, they did not appear to be directly related to Methyleugenol exposure.



ducts lined by highly pleomorphic cells surrounded by abundant collagenous tissue. Cystic degeneration appeared as a focal to multifocal, multilocular, cystic lesion containing an eosinophilic flocculent material. In some of the exposed animals, these lesions were quite large and/or multiple.

Proliferative lesions reported to be associated with chemical exposure in one or both sexes included eosinophilic foci, single to multiple hepatocellular adenomas and carcinomas, cholangiomas and cholangiocarcinomas, and hepatocholangiomas and hepatocholangiocarcinomas. There tended to be a dose-response with respect to the number of neoplasms and malignant potential. Several tumors were diagnosed at the 52-week interim sacrifice in high-dose animals. In addition, greater numbers of metastatic hepatic tumors were noted in high-dose animals.

The criteria used to diagnose the proliferative hepatic lesions were as follows:

Foci of Cellular Alteration (Cell Focus)

1. Localized lesions.
2. Tinctorial variation from surrounding hepatic parenchyma.
3. Foci ranged from less than a hepatic lobule to up to three of four lobules.
4. Hepatocytes merged with adjacent parenchyma without producing compression.
5. Subclassified as clear cell, eosinophilic, basophilic, or mixed cell.



Liver:

The PWG reviewed several slides to confirm the reported treatment-related non-neoplastic and neoplastic lesions. Methyleugenol appeared to be a strong hepatocarcinogen producing neoplastic lesions of both hepatocytes and of the biliary tract.

The PWG confirmed the presence of hepatocellular hypertrophy, oval cell hyperplasia, and cystic degeneration (as diagnosed by the QAP). Focal bile duct atypical hyperplasia was also confirmed in several exposed animals. Bile duct cysts and bile duct hyperplasia (female only) were also reported in exposed animals and were confirmed during the QA review.

Hepatocellular hypertrophy and oval cell hyperplasia were seen as early as the 27-week interim sacrifice and persisted through study termination. Hypertrophy was characterized by increased cell size and cytoplasmic eosinophilia. A strong zonal preference was not observed; however, the SP reported that mid-zonal hepatocytes seemed to be involved chiefly. Oval cell hyperplasia was characterized by the presence of small, oval, slightly basophilic cells which proliferated in rows along the sinusoids next to periportal regions. Atypical bile duct hyperplasia was characterized by the presence of irregularly-shaped, dilated



Hepatocellular Adenoma

1. Usually a discrete lesion which compressed adjacent parenchyma.
2. Well-differentiated cells which were eosinophilic, basophilic or vacuolated.
3. Absence of normal hepatic lobular architecture.
4. Uneven growth patterns.
5. Increased cellular pleomorphism or hypertrophy.

Hepatocellular Carcinoma

1. Distinct trabecular or adenoid pattern.
2. Cells were poorly differentiated or anaplastic.
3. Histologic evidence of local invasiveness or metastasis.
4. Hepatocellular carcinomas can arise within adenomas.

Cholangioma

1. Well-circumscribed, cystic mass containing little connective tissue.
2. Cystic spaces were lined by proliferative bile duct epithelium.
3. Typically were expansive growths.
4. Mitoses were uncommon.

Cholangiocarcinoma

1. Larger neoplasm with abundant connective tissue stroma (scirrhous reaction).
2. Increased cellular pleomorphism and proliferation associated with bile duct epithelium.
3. Increased numbers of mitoses.
4. Local invasion may be observed.



Hepatocholangioma and Hepatocholangiocarcinoma

1. Comprised of neoplastic elements from both hepatocytes and biliary epithelium.
2. Hepatocytes and ductular cells often occurred in the same ductular structure.
3. Both the hepatocellular and biliary epithelium, may be well-differentiated to poorly differentiated depending on biologic behavior.
4. One or the other or both lineages may metastasize in the malignant neoplasms.

Hepatocellular foci, adenoma and carcinoma are believed to represent a spectrum of changes that comprise neoplastic development in the liver. Furthermore, in this study, neoplastic progression of the biliary epithelium and/or hepato-biliary stem cell population appeared to have occurred.

During the PWG examination, several observations were made concerning the proliferative lesions. First, many of the eosinophilic foci and adenomas contained areas of cystic degeneration or vacuolation. Secondly, the distinction between hepatocholangiocarcinomas and hepatocellular carcinomas with glandular patterns were not always clear. However, in general, there was good agreement among the PWG participants and the spectrum of liver lesions confirmed.



Mesothelioma

Slides containing discrepancies concerning the diagnosis of mesothelioma in male animals were shown to the PWG. The presence of mesothelioma was considered as a potential target tissue by the QAP following the QA review.

Following the PWG review, many of the additional mesotheliomas diagnosed by the QAP were confirmed. Apparently, male animals at the 75, 150, and 300 mg/kg dose groups had significantly more mesotheliomas than control males.

NTP criteria was used to diagnose either mesothelial hyperplasia or mesothelioma during the PWG. Mesothelial hyperplasia tends to be a focal thickening of mesothelial cells without stromal or significant villous proliferation. Mesotheliomas may only be a single, papillary projection lined by multiple mesothelial cell layers or appear as florid papilliferous or solid proliferations of neoplastic cells. By convention, all mesotheliomas are considered to be malignant neoplasms.

Most mesotheliomas, in the rat, begin along the mesothelial tunics covering the testis or epididymis, are clonal positive and with time may spread throughout the abdominal cavity. Usually 1-2 mesotheliomas/study may be seen in the historical control data. Up to 4 mesotheliomas



have been reported. In this study a dose-dependent trend was apparent except for the 300 mg/kg males. The fact that no animals survived to study termination and the number of early deaths that occurred may have influenced the final incidence in the 300 mg/kg males.

Bone Marrow:

Representative examples of bone marrow were shown to the PWG because of a number of discrepancies between the SP and QAP and because of the reported increased number of exposed female rats with some type of hyperplastic lesion within the bone marrow.

During the initial study, the SP frequently diagnosed hyperplasia of megakaryocytes, the erythroid cells and myeloid cells. Occasionally, some animals were reported by the SP to have two or more of the hyperplasias. In general, the QAP grouped these lesions under the term hyperplasia because of the inherent difficulty in determining the cell lineage using decalcified, paraffin-embedded sections.

Following the PWG review, there was good agreement to use only hyperplasia, bone marrow. Therefore, the PWG recommended to delete specific references to certain



hyperplasias and add only "Hyperplasia, Bone Marrow". In those few cases where there was total agreement between the SP, QAP, and PWG chairperson of erythroid or myeloid hyperplasia, those cases would remain as diagnosed.

Kidney:

Slides containing discrepancies of proliferative lesions in the male were shown to the PWG. During the study an increased severity of nephropathy was noted, particularly in high-dose males and females. In the males, this was further supported by the increased incidence of secondary lesions related to nephropathy, such as, hyperparathyroidism, tissue mineralization and fibrous osteodystrophy in higher dosed animals. In addition, the QAP diagnosed several more proliferative lesions of the renal tubule in exposed animals. Therefore, the kidney was considered as a potential target tissue. Based on the QAP findings and confirmation by the PWG chairperson, the NTP decided to have a step-section review of kidney sections initiated prior to the PWG.

The criteria used to diagnosed the renal tubule proliferative lesions were as follows:



Renal Tubule, Hyperplasia

1. Hyperplasias maintained their tubular shape and, generally, were not surrounded by a thickened basement membrane indicative of a change associated with advanced nephropathy.
2. Hyperplastic tubules varied in diameter from slightly greater up to approximately 2 to 3 times the diameter of a normal tubule.
3. Epithelial cells usually formed solid clusters and appeared more pleomorphic than normal tubular epithelium.
4. The tinctorial qualities of hyperplastic epithelial cell cytoplasm varied but tended to be more basophilic than the cytoplasm of adjacent renal tubular epithelium.
5. Nuclei tended to be larger with prominent nucleoli.
6. The basement membrane remained intact.
7. Oncocytic hyperplasia was characterized by large, rounded or polygonal epithelial cells which had lightly staining eosinophilic, foamy cytoplasm and round centralized nuclei.

Renal Tubule, Adenoma

1. Adenomas differed from hyperplasia by being larger (usually 5 or more tubular diameters) and generally having a more complex structure with disruption of the tubule basement membrane. Larger adenomas often compressed adjacent parenchyma.
2. Adenomas consisted of several patterns including solid, tubular or papillary patterns.
3. Individual cells generally were more pleomorphic appearing than in hyperplasias.
4. Neoplastic cells, in large adenomas, may be separated by fine bands of connective tissue.
5. Occasional mitoses.
6. Oncocytic adenomas are also characterized by features typical of adenomas (ie. size and compression). However, the cell morphology maintained the foamy eosinophilic appearance with little pleomorphism.



Renal Tubule, Carcinoma

1. Carcinomas were differentiated from adenomas by being much larger and, generally, recognized at gross examination.
2. Hemorrhage, necrosis and locally invasive growth patterns were often prominent features.
3. Cellular anaplasia and/or atypia characterize the neoplastic cells.
4. Patterns of neoplastic cell growth are frequently varied and may consist of several patterns.
5. Despite their sometime aggressive appearance, metastatic sites are rarely found.

Following the PWG review, all of the discrepancies were resolved by the PWG and the trend for increased renal tubule adenomas/carcinomas in exposed animals confirmed. The actual significance of these lesions will be determined following the renal step-section review.

Mammary Gland/Subcutaneous Tissue:

The PWG reviewed several slides of discrepancies involving the diagnosis of fibroadenoma in male animals. During the QA review, additional fibroadenomas were diagnosed by the QAP in exposed male animals and a potential treatment-related trend reported. Most of the additional fibroadenomas were diagnosed in the 75 and 150 mg/kg groups.

Following the PWG review, most of the fibroadenomas reported by the QAP were confirmed by the PWG. Fibroadenomas were characterized as expanding nodules/masses comprised of glandular epithelium (ducts, ductules, and/or alveoli) and fibrous connective tissue. The epithelium, in fibroadenomas,



was generally uniform and single-layered. The connective tissue surrounding the glandular epithelium was prominent and mature appearing. In some fibroadenomas, the connective tissue predominated over the glandular elements.

The number of fibroadenomas in the exposed male groups were within the historical control range but were increased over the controls. The number of fibroadenomas in the 500 mg/kg males was similar to controls; however, these animals did not survive to study termination.

The PWG discussed the increased incidences of fibromas/fibrosarcomas in the exposed male animals, particularly in the 37, 75 and 150 mg/kg groups. Although the SP diagnosed the site of these fibrous neoplasms as the skin, the QAP suggested that they be changed to the subcutaneous tissue. Overall, there was good agreement in the diagnosis of these neoplasms by the SP, QAP and PWG chairperson. Fibromas and fibrosarcomas were diagnosed as solid, expansive neoplasms of fibrous connective tissue. Fibrosarcomas tended to be locally invasive and clearly more anaplastic. Several of the fibromas were, however, considered to be fibroadenomas because of the presence of small amount of glandular epithelium within the tumor.

Because of the importance of two potential positive tumor incidences in male animals (mammary gland fibroadenomas



and subcutaneous tissue fibromas/fibrosarcomas), the PWG decided to further investigate the origin of the fibromas/fibrosarcomas by re-evaluating the study slides and by recutting extra sections from the wet tissue. This would be accomplished as a post-PWG action item.

Salivary Gland:

The PWG confirmed the treatment-related presence of cytologic alteration as reported by the SP in nearly all exposed male and female animals. This lesion was characterized by the loss of the eosinophilic granules within the striated ducts of the submandibular salivary glands. This change was present in the 27-week interim sacrifice animals and persisted throughout the study.

Adrenal Medulla:

Following the QA review, a small increased incidence of adrenal medullary pheochromocytomas were reported by the QAP in the 150 and 300 mg/kg females. The incidence of adrenal medullary hyperplasia, in the exposed females, did not appear related to treatment. No evidence of any chemical affect was observed in the adrenal medulla of males. Therefore, slides involving the diagnosis of pheochromocytoma and/or hyperplasia, primarily in female animals, were shown to the PWG.



The criteria used to diagnose the proliferative lesions of the adrenal medulla were as follows:

Hyperplasia

1. Circumscribed focus of medullary cells that blended with surrounding normal parenchyma.
2. None or minimal compression.
3. Minimal alteration in architecture with cells arranged in packets or solid clusters slightly larger than normal.
4. Minimal to mild alteration in size, shape, and staining qualities of affected cells (nuclei and cytoplasm).

Pheochromocytoma, Benign

1. Well-delineated mass of medullary cells.
2. Minimal to marked compression of surrounding parenchyma.
3. Altered architecture with cells arranged in large solid clusters or thick trabeculae; growth pattern may be variable.
4. Mild to marked alteration in size, shape, and staining qualities of affected cells; cellular atypia and pleomorphism may be marked.

Pheochromocytoma, Malignant

1. Invasion of capsule and periadrenal soft tissue.
2. Metastasis.



Following the PWG review, many of the additional proliferative lesions of the adrenal medulla reported by the QAP were confirmed by the PWG. Even though the number of pheochromocytomas in high-dose females was outside of the historical control data range, the toxicological significance of the increased incidence in high-dose animals was not clear in the absence of any appreciable increase in hyperplasia and essentially a negative trend in the males.

Testes:

The PWG examined several testes for the diagnosis of interstitial cell adenomas and germinal epithelial atrophy. During the study, the SP was inconsistent in the diagnosis of germinal epithelial atrophy when interstitial cell adenomas (unilateral/bilateral) were also present. Apparently, this situation arose due to the necessity of correlating a gross observation (eg. reduced testicular size) to a microscopic lesion. According to the reported data, the incidence of germinal epithelial atrophy was slightly increased in the 150 mg/kg males.

During the PWG review, a few examples of germinal epithelial atrophy were noted in testes with interstitial cell adenomas which were not originally diagnosed and which appeared histologically similar to those cases diagnosed by the SP. Interstitial cell adenomas may be present in testes



reduced in size and in those enlarged. The PWG concurred that germinal epithelial atrophy should not be diagnosed in those testes containing interstitial cell tumors. Furthermore, several PWG participants commented that toxicologic-induced testicular atrophy is difficult to assess at the end of a 2-year study in the F344 rat because of the high incidence of interstitial cell tumors.

Therefore, the PWG recommended that germinal epithelial atrophy be removed as a diagnosis from those testes containing an interstitial cell tumor. The reduction in testis size should be correlated also to the interstitial cell tumor.



MISCELLANEOUS

A number of lesions were examined by the PWG to either confirm their incidence or because a discrepancy existed. In most instances, these lesions represented unusual or diagnostically challenging lesions/neoplasms which were diagnosed either once or only in a few instances from different dose groups, and were not considered to be related to chemical exposure.

~~John Curtis Seely, D.V.M.~~
John Curtis Seely, D.V.M.
Diplomate, American College
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Date June 10, 1998



REFERENCES

Streett CS, Robertson JL and Crissman JW (1988) Morphologic Stomach Findings in Rats and Mice Treated with the H2 Receptor Antagonists, ICI 125,211 and ICI 162,846. Tox Path 16: 299-304.

Hard GC, Iatropoulos MJ, Thake DC, Wheeler D, Tatematsu M, Hagiwara A, Williams GM, and Wilson AGE (1995) Identity and Pathogenesis of Stomach Tumors in Sprague-Dawley Rats Associated With The Dietary Administration of Butachlor. Exp Toxic Pathol 47: 95-105.



**CHAIRPERSON'S REPORT
PATHOLOGY WORKING GROUP (PWG)
OF METHYLEUGENOL (C60991B)
ADMINISTERED VIA ORAL GAVAGE TO
B6C3F1 MICE FOR TWO YEARS PERFORMED
BY BATTELLE COLUMBUS LABORATORIES**

Date OF PWG: May 12-13, 1998

Site of PWG: National Toxicology Program
National Institutes of Environmental Sciences

PWG Participants:

Paul K. Hildebrandt, DVM, Chairperson	PATHCO
James Hailey, DVM	NIEHS
Ronald Herbert, DVM, Ph.D	NIEHS
Abraham Nyska, DVM	NIEHS
Robert Sills, DVM, Ph.D	NIEHS
Michael Ryan, DVM, Ph.D	Battelle Columbus
John Toft, DVM, M.S.	Battelle Columbus
Elias Gaillard, DVM, M.S.	EPL
Gordon Hard, BVSc., Ph.D., DSc.	AHF
Jeffrey Everitt, DVM	CIIT
Spencer Street, VMD	Street & Assoc.
Harold White, MD (consultant)	

Study Pathologist: Dr. John Toft

Reviewing Pathologist: Dr. Elias Gaillard

I. SUMMARY AND CONCLUSIONS

There was a treatment effect in the male and female mice administered methyleugenol via oral gavage for up to two years. The glandular stomach and liver are considered target organs.

A. Glandular Stomach

The lesions in the glandular stomach were characterized by glandular ectasia, glandular atrophy, chronic active inflammation and hyperplasia of the superficial epithelium (mucous neck cells) as well as the glandular epithelium deep in the fundic glands. Several mice of both sexes



had hyperplasia of the neuroendocrine cells. Two high dose male mice had malignant neuroendocrine cell tumors, and a third high dose male mouse had an undifferentiated carcinoma. No females had glandular stomach tumors.

In the low dose of both sexes a higher incidence and severity of glandular ectasia over that of controls was observed.

B. Liver

A profound increase in liver tumors, as well as size and multiplicity of tumors, was seen in both sexes, but especially in females. Hepatoblastomas were seen in high dose males and in all three treatment doses in the females. Over one half of the females in the two higher doses had pulmonary metastatic hepatocellular carcinoma, hepatoblastoma, or both.

Non-neoplastic lesions which occurred in all three doses of treated mice in a dose related fashion were oval cell hyperplasia and hepatocyte hypertrophy. A number of other non-neoplastic lesions were diagnosed in the liver in this study but most were considered to be secondary to, or part of, hepatic neoplasms.

The liver neoplasms in female mice are considered to be the cause of reduced survival in the high dose group.

II. STUDY DESIGN

Male and female B6C3F1 mice were administered methyleugenol via oral gavage for up to two years at the following doses: control, 0 mg/kg, 37 mg/kg, 75 mg/kg or 150 mg/kg. There were 50 mice per group/sex.

III. ANIMAL DISPOSITION SUMMARY

FEMALE MICE

	0 mg/kg	37 mg/kg	75 mg/kg	150 mg/kg
Animals Initially in Study	50	50	50	50
Early Deaths				
Natural Death	14	24	25	35
Moribund Sacrifice	5	7	8	13
Dosing Accident	0	1	1	0
Survivors - Terminal Sacrifice	31	18	16	2
Animals Examined Microscopically	50	50	50	50



III. ANIMAL DISPOSITION SUMMARY (cont'd)

MALE MICE

	0 mg/kg	37 mg/kg	75 mg/kg	150 mg/kg
Animals Initially in Study	50	50	50	50
Early Deaths				
Natural Death	6	8	7	4
Moribund Sacrifice	5	6	5	9
Accidentally Killed	0	0	1	1
Dosing Accident	0	0	0	1
Missing	1*	0	0	0
Survivors - Terminal Sacrifice	38	36	37	35
Animals Examined Microscopically	49	50	50	50

*Male mouse number 50 (control) was accidentally removed from the room during a cage change. The reason for removal was entered as missing.

IV. CONDUCT OF THE PWG

Prior to the PWG, the chairperson reviewed the pathology incidence tables, the study pathologist's narrative, the report prepared by the reviewing pathologist, microscopic slides of potential target organs, and selected lesions with discrepancies in diagnoses between the study pathologist (SP) and reviewing pathologist (RP).

Potential target organs which were examined for all tumor and non-tumor diagnoses for all animals in all groups are listed as follows:

Male Mice

Liver
Stomach, glandular
Spleen
Bone Marrow
Lung

Female Mice

Liver
Stomach, glandular
Spleen
Bone Marrow



The following organs/tissues were examined for all animals in all groups for specific lesions:

Male Mice

Adrenal Gland - Hematopoietic
cell proliferation
Thymus - Lymphoma

Female Mice

Adrenal Gland - Hematopoietic
cell proliferation
Heart - Thrombosis

The following organs from the sex indicated were reviewed when the specific diagnoses listed were present:

Male Mice

Lung - Infiltration, cellular, lymphocyte
Lung - Alveolus-Infiltration, cellular, lymphocyte
Nose - Inflammation, suppurative
Nose - Nasolacrimal Duct-Inflammation, suppurative
Tooth - Malformation
All tumors in all organs

Female Mice

Lung - Infiltration, cellular, lymphocyte
Lung - Alveolus-Infiltration, cellular, lymphocyte
Nose - Inflammation, suppurative
Nose - Nasolacrimal Duct-Inflammation, suppurative
Tooth - Malformation
All tumors in all organs

At the PWG the chairperson led a short discussion in the form of introductory remarks which included:

- 1) Study design
- 2) Survival data
- 3) Gross necropsy lesions

A. Glandular Stomach

The chairperson had photographed a number of glandular stomachs and livers with representative treatment related lesions. The photomicrographs were projected and shown to the PWG to review the histology of the glandular stomach and to introduce the types of lesions present. Topography and appropriate diagnostic nomenclature for the stomach lesions were reviewed and discussed to select terms that best depicted the lesion and to maintain consistency between lesions encountered in the mouse study as well as between the mouse and rat studies.



The study pathologist used the topographical terms stomach gland and stomach gland, glands to refer to lesions in the glandular region of the gastric mucosa. In addition, the RP used the terms stomach, glandular-epithelium to refer to lesions in the epithelium of the gastric mucosa above the parietal cells, and stomach glandular-epithelium, glands to refer to lesions in the epithelium of the deep glands of the gastric mucosa. The PWG recommended that one topography, stomach gland, be used for all lesions in the glandular stomach for consistency within the study and between the mouse and rat studies.

There was little disagreement between the SP and RP regarding ectasia of the mucosal glands in the stomach of mice in this study. The RP often had additional diagnoses such as glandular hyperplasia, mucosal atrophy and chronic active inflammation in stomachs with glandular ectasia.

A group of slides representing non-neoplastic and neoplastic lesions of the stomach were presented to the PWG for examination. These slides were from control as well as treated mice of both sexes. Following examination of these stomachs, the PWG characterized the lesions as follows.

Ectasia of mucosal glands was characterized by dilatation of the gland and it most often contained small amounts of necrotic epithelial cells and/or cellular debris. The epithelial lining of these ectatic glands was generally degenerate, flattened and, in some glands, a few necrotic cells were present. In control mice an occasional mucosal gland was observed to be dilated (ectatic) but had only minimal altered epithelial lining and contained essentially no cellular debris. There was a definite compound effect regarding glandular ectasia of the glandular stomach of treated mice.

Atrophy of the glandular stomach was characterized by a reduction in or loss of chief cells (basophilic cells) and parietal cells (eosinophilic cells) in more severe cases. Marked atrophy was characterized by a reduction in the thickness of the gastric mucosa.

Inflammation, active chronic was characterized by focal accumulation of mononuclear cells with a few neutrophils located in the lamina propria and submucosa. A very small focus of such inflammatory cells was occasionally observed in a control mouse, but the size and number of such foci were definitely increased in treated mice.

Hyperplasia of the epithelium in the glandular stomach occurred in two different cell types. There was hyperplasia of the more columnar epithelium, or the surface of the gastric mucosa (mucous neck cells). This change was diagnosed as stomach glandular hyperplasia and was characterized by an increased number of such cells which were usually larger and more eosinophilic than normal and somewhat pleomorphic. This proliferation of epithelial cells often extended down the gastric gland and occasionally appeared to be associated with an ectatic lesion at the blind end of the affected gland.



The second type of hyperplasia was characterized by proliferation of chief cells near the base of the fundic glands. Hyperplasia of the glandular epithelium in the base of the fundic glands was characterized by an increase in small fundic glands. The epithelium of these glands contained abundant light eosinophilic to basophilic cytoplasm and a distinct round nucleus.

In several stomachs of treated mice, there was a focus of proliferating cells that had indistinct cytoplasmic borders and a round to slightly oblong heavily basophilic stippled nucleus. These cells were in between fundic glands near the muscularis mucosa. These cells had been diagnosed as atypical hyperplasia of glandular epithelium; however, it was the PWG opinion these were neuroendocrine cells. All stomachs with a previous diagnosis of atypical hyperplasia were reviewed and considered by the PWG to be neuroendocrine cell hyperplasia.

The PWG reviewed the 4 neoplasms diagnosed in glandular stomachs of male mice. No neoplasm had been diagnosed in the glandular stomach of female mice. The PWG was of the opinion that two of these neoplasms were malignant neuroendocrine cell tumors. One was an undifferentiated carcinoma. The fourth tumor was considered to be marked glandular hyperplasia with cystic invagination into the submucosa.

The PWG examined a set of low dose male and female glandular stomachs and agreed a compound effect was present in these dose groups. The effect was an increased incidence and severity of ectasia over that present in controls.

The PWG agreed that a dose-related compound effect, was present in the glandular stomach of both male and female mice. There appeared to be a greater effect in male mice although the reduced survival of female mice may have influenced this finding.

The gastric lesions consisted of chronic active inflammation, glandular ectasia, mucosal atrophy and epithelial and glandular hyperplasia. Neuroendocrine cell hyperplasia and malignant neuroendocrine cell tumors occurred primarily in high dose mice.

There were no treatment related lesions in the forestomach.

B. Liver

A dose related treatment effect, in the liver, was identified by the SP and confirmed by the RP. Proliferative liver lesions in the form of oval cell hyperplasia, eosinophilic foci, hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastomas occurred at a higher incidence in treated mice compared to controls. Also, the size and multiplicity of liver tumors, especially hepatocellular adenoma and hepatoblastomas in female mice, were greater in treated mice compared to controls.



The chairperson projected a series of photomicrographs that illustrated the oval cell hyperplasia and portal hepatocellular hypertrophy that had been diagnosed in treated mice of both sexes.

The PWG examined a series of liver slides with representative lesions. There were only a few discrepancies between the study pathologist and reviewing pathologist regarding mice with a liver tumor versus non-tumor. These discrepancies were included in the liver slides reviewed.

The PWG recognized that oval cell hyperplasia and hepatocyte hypertrophy was present only in treated mice. The oval cells were small with indistinct cytoplasmic borders and contained an oblong deeply basophilic nucleus. The cells were more numerous near the portal triad and extended along sinusoids toward the central vein. A suspicion of differentiation toward bile duct epithelium was observed in an occasional liver lobe. Several livers from control mice were examined and no oval cell hyperplasia or hepatocyte hypertrophy was recognized. This coincided with the observations of the SP and RP. The question of *Helicobacter* infection was raised because oval cell hyperplasia and hypertrophy of hepatocytes are common lesions in mice with this organism; however, since controls were not affected, a compound effect is considered to be the likely cause. Also, silver stains performed on random sampling of affected livers were negative. A number of livers had been diagnosed with bile duct hyperplasia. The PWG agreed that most of these lesions were, in fact, oval cell hyperplasia with a few cells differentiated toward biliary epithelial cells.

The criteria utilized by the PWG in diagnosing proliferating hepatic lesions were those published by Maronpot¹, and are briefly described as follows:

1. Foci of Cellular Alteration

Foci of cellular alteration are characterized by focal clusters of hepatocytes that have altered cytoplasmic tinctorial properties. Based on cytoplasmic staining with hematoxylin and eosin, they have been classified as; basophilic, eosinophilic, clear cell, or mixed type. Cellular atypia is generally absent although cells may be smaller or larger than unaffected hepatocytes. Foci of cellular alteration vary in size from a few cells to lesions that occupy several hepatic lobules. The majority, however, are smaller than one hepatic lobule, and there is generally sharp demarcation between foci and surrounding liver. There is little or no alteration of normal hepatic lobular architecture within foci; however, the architecture of the cords is often distorted. Large foci or those with hypertrophied cells or vascular ectatic lesions sometimes distort or compress surrounding hepatic cords. Such compression is not a prominent feature.

2. Hepatocellular Adenoma

Hepatocellular adenomas are generally round lesions usually occupying an area greater than one liver lobule. A portion of the circumference is usually sharply demarcated from



the surrounding liver tissue by either compression of normal liver or lack of continuity between the cords of the nodule and those of the adjacent unaffected liver. The cords of an adenoma are often perpendicular to or impinge obliquely on the cords of the adjacent unaffected liver. Within the tumor there is a loss of normal lobular architecture and areas of cellular atypia are often present. Hepatocytes within the nodule are usually eosinophilic with features such as cytoplasmic vacuolization. The hepatic cords, when discernable, are arranged haphazardly and frequently are more than one cell layer thick. Adenomas rarely contain portal triads although, occasionally, triads may be present at the periphery, the result of envelopment as the adenoma grows by expansion.

3. Hepatocellular Carcinoma

Hepatocellular carcinomas are usually not as well-demarcated as adenomas and generally have areas where cell proliferation assumes a trabecular or adenoid pattern. Cellular features generally are more anaplastic; however, fairly well-differentiated hepatocytes may be present in some hepatocellular carcinomas. Metastasis to the lung can occur usually in livers that have a tumor with a high degree of anaplasia.

4. Hepatoblastoma

Hepatoblastomas were generally well-demarcated from adjacent liver tissue and were composed of poorly differentiated, small, slightly elongated basophilic cells, often aligned radially, around numerous, small blood vessels. Mitotic figures were quite common. Metastasis to the lung occurred in several cases. In some cases there were both hepatocellular carcinoma and hepatoblastoma metastatic foci in the lung. Hepatoblastoma generally occurs with hepatocellular adenoma or carcinoma, as was observed in almost every case in this study.

A number of lesions in the liver were diagnosed which were considered to be secondary to the large liver tumor present. These lesions included necrosis, thrombosis, mineralization, hemosiderosis, fibrosis and inflammation, acute/chronic. The PWG was of the opinion that most of these lesions were a part of or secondary to the liver tumor that was present, and an additional diagnosis of these lesions was not necessary.

C. Lung

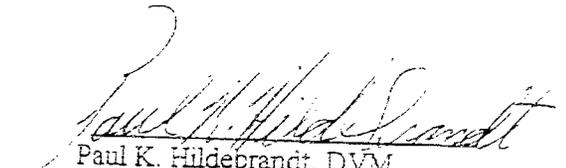
There was a slight increase in alveolar bronchial tumors in the lungs of the male mice over that of the controls. A compound effect is questioned because the increase in pulmonary tumors did not occur in a dose related fashion and there was no increase in alveolar bronchial hyperplasia. There was no increase in pulmonary tumors in the female mice.



D. Miscellaneous

Bone marrow hyperplasia and hematopoietic proliferation of the spleen, liver and adrenal glands were diagnosed in all dose groups, including controls. There was a higher incidence in treated mice but it was the PWG opinion that this increased hematopoietic proliferation was a secondary effect due to the increased necrosis and inflammation associated with the large and multiple liver tumors in treated mice, especially females.

A set of slides with interesting and unusual lesions in non target tissues, along with a few slides that had lesions with a discrepancy in diagnosis were presented to the PWG for review. Results are listed in the Appendix.


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D 01



REFERENCE

Maronpot, R. R., et al. (1987). Liver Lesions in B6C3F1 Mice: The National Toxicology Program, Experience and Position, Arch Toxicol. Supp 10:10-26.

TR-491 Methyl Eugenol
Pathology Tables

RATS

A. 27 Week Interim Sacrifice

P03 - Incidence Rates of Non-Neoplastic Lesions -

P05 - Incidence Rates of Neoplasms by Anatomic Site (systemic lesions abridged)

B. 52 Week Interim Sacrifice

P03 - Incidence Rates of Non-Neoplastic Lesions -

P05 - Incidence Rates of Neoplasms by Anatomic Site (systemic lesions abridged)

C. Core Study

P03 - Incidence Rates of Non-Neoplastic Lesions

P05 - Incidence Rates of Neoplasms by Anatomic Site (systemic lesions abridged)

P08 - Statistical Analysis of Primary Tumors

D. Stop Study

P08 - Statistical Analysis of Primary Tumors

MICE

Core study

P03 - Incidence Rates of Non-Neoplastic Lesions

P05 - Incidence Rates of Neoplasms by Anatomic Site (systemic lesions abridged)

P08 - Statistical Analysis of Primary Tumors

**TR-491 Methyl Eugenol
Pathology Tables**

RATS

A. 27 Week Interim Sacrifice

P03 - Incidence Rates of Non-Neoplastic Lesions -

P05 - Incidence Rates of Neoplasms by Anatomic Site (systemic lesions abridged)

TP Experiment-Test: 05187-09
Study Type: CHRONIC
Route: GAVAGE

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
METHYLEUGENOL

Report: PEIRPT03
Date: 07/20/98
Time: 08:39:26

27 WEEK SSAC

Facility: Battelle Columbus Laboratory

Chemical CAS #: 93-15-2

Lock Date: 09/17/96

Cage Range: All

Reasons For Removal: 25017 Scheduled Sacrifice

Removal Date Range: 08/09/94 - 08/10/94

Treatment Groups:	Include 002	0	MG/KG
	Include 010	300	MG/KG
	Include 001	0	MG/KG
	Include 009	300	MG/KG

a Number of animals examined microscopically at site and number of animals with lesion

Report: PEIRP03
Date: 07/20/98
Time: 08:39:26

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
METHYLEUGENOL

NTP Experiment-Test: 05187-09
Study Type: CHRONIC
Route: GAVAGE

FISCHER 344 RATS FEMALE 0 MG/KG 300 MG/KG

DISPOSITION SUMMARY

Animals Initially In Study	60	60
Scheduled Sacrifice	5	5
Early Deaths		
Survivors		
Animals Examined Microscopically	5	5

ALIMENTARY SYSTEM

Liver	(5)	(5)
Basophilic Focus		2 (40%)
Clear Cell Focus		2 (40%)
Hepatodiaphragmatic Nodule	1 (20%)	
Mixed Cell Focus		3 (60%)
Hepatocyte, Hypertrophy		5 (100%)
Oval Cell, Hyperplasia		5 (100%)
Pancreas	(5)	(5)
Acini, Atrophy		1 (20%)
Salivary Glands	(5)	(5)
Submandibular Gland, Cytoplasmic Alteration		5 (100%)
Stomach, Glandular	(5)	(5)
Atrophy		5 (100%)
Neuroendocrine Cell, Hyperplasia		1 (20%)

CARDIOVASCULAR SYSTEM

None

ENDOCRINE SYSTEM

Islets, Pancreatic	(5)	(5)
Hyperplasia	1 (20%)	

GENERAL BODY SYSTEM

None

a Number of animals examined microscopically at site and number of animals with lesion

Report: PRIRPT03
 Date: 07/20/98
 Time: 08:39:26

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
 METHYLEUGENOL

NTP Experiment-Test: 05187-09
 Study Type: CHRONIC
 Route: GAVAGE

FICHER 344 RATS FEMALE

0 MG/KG 300
 MG/KG

GENITAL SYSTEM

Ovary	(5)	(5)
Cyst	1 (20%)	
Uterus	(5)	(5)
Hyperplasia, Cystic	1 (20%)	

HEMATOPOIETIC SYSTEM

Bone Marrow	(5)	(5)
Hyperplasia	4 (80%)	
Lymph Node	(1)	
Erythrophagocytosis	1 (100%)	

INTRUMENTARY SYSTEM

None

MUSCULOSKELETAL SYSTEM

None

NERVOUS SYSTEM

None

RESPIRATORY SYSTEM

Lung	(5)	(5)
Inflammation, Granulomatous	1 (20%)	

SPECIAL SENSES SYSTEM

None

URINARY SYSTEM

None

a Number of animals examined microscopically at site and number of animals with lesion.

Report: PEIRPT03
Date: 07/20/98
Time: 08:39:26

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
METHYLEUGENOL

NTP Experiment-Test: 05187-09
Study Type: CHRONIC
Route: GAVAGE

FISCHER 344 RATS MALE	0 MG/KG	300 MG/KG
DISPOSITION SUMMARY		
Animals Initially In Study	60	60
Scheduled Sacrifice	5	5
Early Deaths		
Survivors		
Animals Examined Microscopically	5	5
ALIMENTARY SYSTEM		
Intestine Large, Rectum	(5)	(5)
Parasitic Metazoan	1 (20%)	(5)
Liver	(5)	3 (60%)
Basophilic Focus		3 (60%)
Eosinophilic Focus		5 (100%)
Hepatodiaphragmatic Nodule	1 (20%)	5 (100%)
Mixed Cell Focus		5 (100%)
Hepatocyte, Hypertrophy		(5)
Oval Cell, Hyperplasia		2 (40%)
Pancreas		1 (20%)
Acinus, Atrophy		(5)
Duct, Hyperplasia		(5)
Salivary Glands		5 (100%)
Submandibular Gland, Cytoplasmic Alteration		(5)
Stomach, Glandular Atrophy		5 (100%)
CARDIOVASCULAR SYSTEM		
Heart	(5)	(5)
Myocardium, Degeneration	2 (60%)	2 (40%)
ENDOCRINE SYSTEM		
Islets, Pancreatic Hyperplasia	(5)	(5)
GENERAL BODY SYSTEM		
None	1 (20%)	

a Number of animals examined microscopically at site and number of animals with lesion

Report: P RPT03
 Date: 07/29/98
 Time: 08:39:26

INCIDENCE RATES OF NONNEOPLASTIC LESIONS BY ANATOMIC SITE (a)
 METHYLEUGENOL

NTP Experiment-Test: 05187-09
 Study Type: CHRONIC
 Route: GAVAGE

FISCHER 344 RATS MALE	0 MG/KG	300 MG/KG
GENITAL SYSTEM		
Preputial Gland Inflammation, Chronic	(5) 1 (20%)	(5) 1 (20%)
HEMATOPOIETIC SYSTEM		
Bone Marrow Hyperplasia	(5) 1 (20%)	(5) 1 (20%)
INTRUMENTARY SYSTEM		
None		
MUSCULOSKELETAL SYSTEM		
None		
NERVOUS SYSTEM		
None		
RESPIRATORY SYSTEM		
None		
SPECIAL SENSES SYSTEM		
None		
URINARY SYSTEM		
Kidney Nephropathy	(5) 4 (80%)	(5) 5 (100%)

a Number of animals examined microscopically at site and number of animals with lesion

Report: PEIRPT05
Date: 07/20/98
Time: 08:39:36

INCIDENCE RATES OF NEOPLASMS BY ANATOMIC SITE (SYSTEMIC LESIONS ABRIDGED) (a)

TP Experiment-Test: 05187-09
Study Type: CHRONIC
Route: GAVAGE

METHYLENOL

27 WEEK SSAC

Facility: Battelle Columbus Laboratory

Chemical CAS #: 93-15-2

Lock Date: 09/17/96

Cage Range: All

Reasons For Removal: 25017 Scheduled Sacrifice

Removal Date Range: 08/09/94 - 08/10/94

Treatment Groups:	Include 002	0	MG/KG
	Include 010	300	MG/KG
	Include 001	0	MG/KG
	Include 009	300	MG/KG

a Number of animals examined microscopically at site and number of animals with lesion

NTP Experiment-Test: 05187-09 INCIDENCE RATES OF NEOPLASMS BY ANATOMIC SITE (SYSTEMIC LESIONS ABRIDGED) (a) Report: PEIRPT05
Study Type: CHRONIC Date: 07/20/98
Route: GAVAGE METHYLEUGENOL Time: 08:39:36

FISHER	344 RATS FEMALE	0 MG/KG	300 MG/KG
DISPOSITION SUMMARY			
Animals Initially in Study	60	60	60
Scheduled Sacrifice	5	5	5
Early Deaths			
Survivors			
Animals Examined Microscopically	5	5	5

ALIMENTARY SYSTEM
None

CARDIOVASCULAR SYSTEM
None

ENDOCRINE SYSTEM
None

GENERAL BODY SYSTEM
None

GENITAL SYSTEM
None

HEMATOPOLETIC SYSTEM
None

INTEGUMENTARY SYSTEM
None

Report: PETRPT05
Date: 07/20/98
Time: 08:39:36

NTP Experiment-Test: 05187-09 INCIDENCE RATES OF NEOPLASMS BY ANATOMIC SITE (SYSTEMIC LESIONS ABRIDGED) (a)
Study Type: CHRONIC METHYLEUGENOL
Route: GAVAGE

FISCHER 344 RATS FEMALE 0 MG/KG 300 MG/KG

MUSCULOSKELETAL SYSTEM

None

NERVOUS SYSTEM

None

RESPIRATORY SYSTEM

None

SPECIAL SENSES SYSTEM

None

URINARY SYSTEM

None

Report: PEIRP05
Date: 07/20/98
Time: 08:39:36

NTP Experiment-Test: 05187-09 INCIDENCE RATES OF NEOPLASMS BY ANATOMIC SITE (SYSTEMIC LESIONS ABRIDGED) (a)
Study Type: CHRONIC
Route: GAVAGE
METHYLEUGENOL

FISCHER 344 RATS MALE	0 MG/KG	300 MG/KG
DISPOSITION SUMMARY		
Animals Initially in Study	60	60
Scheduled Sacrifice	5	5
Early Deaths		
Survivors		
Animals Examined Microscopically	5	5

ALIMENTARY SYSTEM

None

CARDIOVASCULAR SYSTEM

None

ENDOCRINE SYSTEM

None

GENERAL BODY SYSTEM

None

GENITAL SYSTEM

None

HEMATOPOIETIC SYSTEM

None

INTEGUMENTARY SYSTEM

None

NTP Experiment Test: 05187-09 INCIDENCE RATES OF NEOPLASMS BY ANATOMIC SITE (SYSTEMIC LESIONS ABRIDGED) (a) Report: PEIRPT05
Study Type: CHRONIC METHYLEGENOL Date: 07/20/98
Route: GAVAGE Time: 08:39:36

FISCHER J44 RATS MALE 0 MG/KG 100 MG/KG

MUSCULOSKELETAL SYSTEM

None

NERVOUS SYSTEM

None

RESPIRATORY SYSTEM

None

SPECIAL SENSES SYSTEM

None

URINARY SYSTEM

None