

8EHQ-0402-14885



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April 5, 2002

ML 58008

TSCA Document Control Office (7408)
Office of Pollution Prevention and Toxics
Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington DC 20460

Attention: TSCA 8(e) Coordinator

RE: 8EHQ Number: 8EHQ0301-14885 - Supplemental Information
2-Phenylethyl Alcohol (CAS No. 60-12-8) - Cell Proliferation in Lungs of Mice
Administered Intraperitoneal Doses

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Dear Sir or Madam:

In March 2001, Lyondell Chemical Company (Lyondell) submitted information to EPA in accordance to Section 8(e) of the Toxic Substances Control Act (TSCA) and EPA's 1991 Section 8(e) Reporting Guide. In that correspondence, Lyondell reported the receipt of information, which to Lyondell's knowledge, was the first report of alveolar cell proliferation in the mouse lung following intraperitoneal administration of 2-Phenylethyl alcohol (CASRN 60-12-8). At the time of our submission, referenced as 8EHQ0301-14885, only preliminary results were available. Though a final report has not yet been obtained by Lyondell, we have received an abstract of the study as well as a poster made available at the Society of Toxicology (SOT) 2002 Annual Meeting in March 2002. Therefore, Lyondell is hereby submitting the abstract and poster as supplemental information to last year's Section 8(e) submission.

As indicated in the original Section 8(e) submission, Lyondell will forward the final report for this study when it becomes available.

Should you have any questions or require additional details, please do not hesitate to call me at 713/309-2136. I may also be reached by facsimile at 713/951-1574 or by e-mail at patrick.gibson@lyondell.com.

Sincerely,

Patrick L. Gibson
Product Safety Specialist - Regulatory
Corporate TSCA Coordinator
Lyondell Chemical Company

Enclosure

Contain NO CBI



8EHQ-01-14885



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MECHANISM of TUMOUR FORMATION in MICE by STYRENE: Induction of Cell Proliferation by Styrene Metabolites

R. Landsiedel, W. Kaufmann, W. Meilert, H.D. Hoffmann BASF Aktiengesellschaft, Product Safety, 67056 Ludwigshafen, Germany

EXPOSURE
 Production 2 x 10⁷ lpm/year for polymers and copolymers
 Environment 1 - 3 µg/m³ Workplace exposure limit 80 mg/m³ (20 ppm)
 Food 0.2 - 1.2 µg/kg/day Cigarettes 0.1 - 10 µg/cigarette

CANCER
 Rat: No tumor induction by chronic inhalation of up to 1000 ppm
 Mice: Lung tumors by chronic inhalation of 20 ppm and above
 Epidemiological: Chest data show no persuasive evidence of styrene induced cancer in humans.

MODE of ACTION
 • Not or only slightly mutagenic
 • Little DNA-binding, but no differences between low and high and between rats and mice
 • Genetically unlikely to be involved in lung tumor formation
 • Short-term styrene inhalation caused Clara cell toxicity and proliferation in mice lungs
 • No such effect was found in lungs of rats
 • Dependent on Cytochrome P450 metabolism
 • Induction of cell proliferation in the lungs of mice is a major cause of tumor formation by styrene rather than a genotoxic effect.
 • Processes leading to cell proliferation in the lungs of mice but not rats are not yet completely understood.

STYRENEOXIDE
 • Styrene is metabolized to styreneoxide
 • Styreneoxide is assumed to cause acute genotoxicity
 • Lung burdens of styreneoxide mice > rats > humans, but
 Styreneoxide can not be seen as the genotoxic agent.

METABOLISM
 • 4-Oxidation of styrene is an important pathway in mice, but not in rats and humans.
 • The metabolism leads to phenylacetaldehyde and phenylacetic acid.
 • Local metabolism in Clara cells may be decisive for tumor formation.



QUESTIONS
 Are p-oxidized metabolites, predominantly formed by mice, the ultimate toxicants?
 Do they induce cell proliferation in the lung, and thus promote tumor formation in the lungs of mice?

METHODS

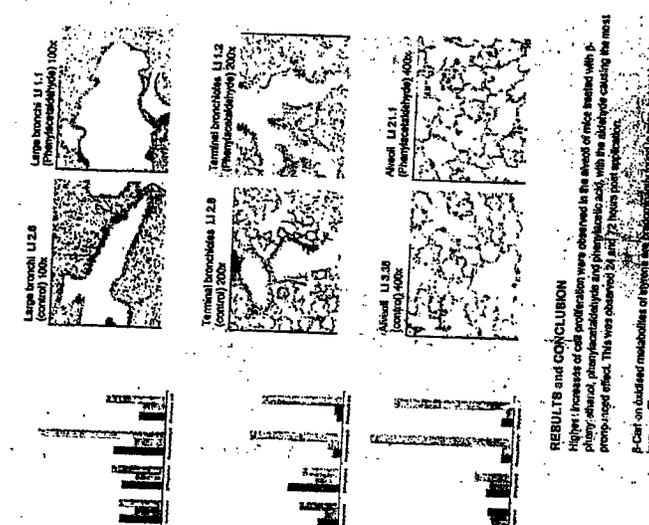
We tested styrene and five of its metabolites for induction of cell proliferation in the lungs of CD-1 mice.

1-24
 • p-oxidized styrene
 • p-oxidized styrene
 • p-oxidized styrene
 • p-oxidized styrene
 • p-oxidized styrene

1-72
 The test substances were administered to mice for 24 hours. The cell proliferation in the lungs was measured by the ³H-thymidine incorporation method.

1-72
 The cell proliferation in the lungs was measured by the ³H-thymidine incorporation method.

1-72
 The cell proliferation in the lungs was measured by the ³H-thymidine incorporation method.



RESULTS CONCERN PROLIFERATION
 Higher increases of cell proliferation were observed in the alveoli of mice treated with p-oxidized styrene, phenylacetaldehyde, and phenylacetic acid, with the aldehyde causing the most pronounced effect. This was observed in mice 24 hours post-exposure.
 p-Clair on oxidized metabolites of styrene...
 These metabolites are the most likely candidates for the observed effects in the lung. This could considerably contribute to the...
 explain the differences between mice and rats...
 assessment of risks in humans.

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These results add biological plausibility to our previous epidemiological observations linking p, p'-DDE and breast cancer aggressiveness.

1445 MECHANISM OF P53 ELEVATION BY BUTADIENE DIEPOXIDE IN HUMAN FIBROBLASTS.

P. M. Muganda, S. Y. Seemanapalli and M. Starks. *Biological Sciences, Southern University, Baton Rouge, LA.* Sponsor: F. Spencer.

The environmental chemical butadiene is prevalent in petrochemical industrial areas, and is a known mutagen and suspected human carcinogen. The molecular mechanisms of butadiene toxicity, however, are not yet well understood, though the cellular effects of this compound are mediated through its metabolites. Since the tumor suppressor p53 protein responds to cellular stress, and mediates cellular responses to environmental stress and DNA damage, we investigated whether p53 levels were affected in human embryonic lung fibroblasts exposed to butadiene diepoxide, the most genotoxic metabolite of butadiene. We found that under experimental conditions where glyceraldehyde-3-phosphate dehydrogenase (G3PDH) levels remain unchanged after western blot analysis, butadiene diepoxide was effective in elevating p53 levels in exposed fibroblasts at concentrations as low as 1 micromolar. Levels of p53 were elevated 10 fold at 1 micromolar butadiene diepoxide concentration, and continued to increase as the concentration of butadiene diepoxide increased. Levels of p53 were maximal when butadiene diepoxide concentrations of 200 micromolar were utilized, and at this point p53 levels were elevated approximately 40 fold as compared to control fibroblasts. Further investigation by pulse chase analysis revealed that p53 stability was mainly responsible for the elevated p53 levels in butadiene diepoxide exposed fibroblasts. Our data also revealed that levels of acetylated p53 at lysine 382 increased in correlation to the elevated p53 levels in butadiene diepoxide exposed cells. Since p53 acetylation has been found to be important in stabilizing and regulating p53 function in response to genotoxic stress in other systems, our results suggest that p53 acetylation at lysine 382 may play a similar role in human fibroblasts exposed to butadiene diepoxide.

1446 TUMOR FORMATION IN MICE BY STYRENE: INDUCTION OF CELL PROLIFERATION IN THE LUNG BY STYRENE METABOLITES.

R. Landseidel, W. Kaufmann, W. Mellert and H. D. Hoffmann. *Department of Products Safety, BASF, Ludwigshafen, Germany.* Sponsor: R. Bahnmann.

Styrene is a high production volume chemical (approx. 2×10^7 tons/year) used as a monomer for plastic productions. Mice develop lung tumors when exposed chronically to styrene. The relevance of these findings for humans is under investigation. Epidemiological data provide no evidence for a carcinogenic potential in humans. Styrene is metabolized to styreneoxide. This electrophilic metabolite is assumed to cause genotoxicity and consequently lung tumors in mice. Research on the metabolism, kinetics and genotoxic effects of styreneoxide in mice and rats did not disclose a clear-cut model for tumor formation depending predominantly on styreneoxide. Thus styreneoxide cannot be seen as the only ultimate toxicant. Recent mechanistic studies demonstrated that the induction of cell proliferation in the lungs of mice is a major cause of tumor formation by styrene rather than a genotoxic effect. We tested styrene and five of its metabolites (α -phenylethanol, β -phenylethanol, phenylacetaldehyde, acetophenone, phenylacetic acid) for induction of cell proliferation in the lungs of CD-1 mice and Fisher rats. Test substances were administered i.p. The cell proliferation in the lungs was quantified by the S-phase response (BrdU labelling index). It was determined 24 and 72 hours post application in large and medium bronchi, terminal bronchi and alveoli. Highest increases of cell proliferation were observed in the alveoli of mice treated with β -phenylethanol, phenylacetaldehyde and phenylacetic acid, with the aldehyde causing the most pronounced effect. This was observed 24 and 72 hours post application. Interestingly the oxidation at the β -carbon of styrene is an important pathway in mice, but not in rats. This metabolism leads to phenylacetaldehyde and phenylacetic acid, the most potent inducer of cell proliferation in this study. A considerable contribution of these metabolites to the tumor formation by styrene exposure would explain the differences between mice and rats and may provide a better basis for the assessment of risks in humans.

1447 FUNCTIONAL CHARACTERIZATION OF C190T POLYMORPHISM IN HUMAN N-ACETYLTRANSFERASE 2 (NAT2) GENE.

Y. Zhu, M. A. Doll and D. W. Hein. *Pharmacology and Toxicology, University of Louisville, Louisville, KY.*

N-acetyltransferase 2 (NAT2) is an important phase II enzyme, which catalyzes N-acetylation (detoxification) and O-acetylation (activation) of many drugs and environmental carcinogens. Genetic polymorphisms in the NAT2 gene have been asso-

ciated with... Single nucleotide polymorphisms (SNPs) have been identified in the human NAT2 coding region. Their effects on NAT2 expression, stability, and catalytic activity have been investigated in our laboratory. A new allele NAT2*19 recently was identified. This variant allele possessing the C190T (R64W) SNP was cloned and expressed in yeast (*Schistosaccharomyces pombe*). Substantial reduction in expression of NAT2 immunoreactive protein and protein stability were observed for NAT2*19 (half-life=2.71 min) when compared with the reference rapid acetylator allele NAT2*4 (half-life=36.4 min). No significant differences in mRNA expression or transformation efficiency were observed following Northern and Southern blotting. The enzymatic activities for N-acetylation of two arylamine carcinogens (2-aminofluorene, 4-aminobiphenyl), and a sulfonamide drug (sulfamethazine) were over 100-fold lower for NAT2 19 compared to reference NAT 4. In addition, activity for O-acetylation of the heterocyclic amine carcinogen 2-amino-1-methyl-6-phenylimidazo [4, 5-b] pyridine was 24-fold lower. The results show that NAT2*19 encodes slow acetylator phenotype for both N- and O-acetylation. Our findings define more clearly the effects of individual SNPs on human NAT2 expression, stability and catalytic activity. This work is partially supported by USPHS grant CA34627 from the National Cancer Institute.

1448 A NOVEL REGULATORY MECHANISM FOR TYROSINE HYDROXYLASE.

R. R. Vaillancourt and N. A. Sachs. *Department of Pharmacology & Toxicology, University of Arizona College of Pharmacy, Tucson, AZ.*

Tyrosine Hydroxylase (TH) is regulated by the reversible phosphorylation of serines 8, 19, 31 and 40. The physiological significance of phosphorylation of the serines remains enigmatic, as pan phosphorylation is not obligatory for catecholamine biosynthesis. The purpose of our study was to characterize the physiological relevance of the heterogeneous phosphorylation of TH by identifying novel interacting proteins. Serine 19 is arguably unique due to its requirement of 14-3-3 binding after phosphorylation for optimal enzyme activity, although it has been suggested that phosphorylated serine 40 also binds 14-3-3. 14-3-3 proteins are prevalent in mammalian brain and isoforms of 14-3-3 serve diverse functions including the regulation of intracellular signal transduction cascades by serving as a scaffold between proteins. To characterize proteins that interact with TH after phosphorylation on serine 19, this amino acid was mutated to alanine (THS19A), since it was reported that a serine to alanine mutation promotes a stable interaction between proteins. For the identification of proteins that interact with TH, THS19A was used as the bait in the yeast two-hybrid system and two cDNA clones were identified, consisting of amino acids 147-262 of mouse-derived PITSLRE. PITSLRE is a serine/threonine kinase and characterization of the corresponding human cDNA, in the form of a GST fusion protein, encoding amino acids 1-373 of a splice variant of the isoform of human PITSLRE demonstrates an interaction between TH and GST-PITSLRE (1-373). Additionally, *in vitro* transcribed and translated TH interacts with full-length FLAG-PITSLRE immunoprecipitated from COS-7 cells. TH does not serve as a substrate for PITSLRE in an *in vitro* kinase assay. However, PITSLRE prevents the interaction between TH and 14-3-3 in HEK 293 cells through a mechanism, which remains to be characterized. It appears that PITSLRE may serve as a negative regulator of TH activity and therefore, possibly catecholamine biosynthesis.

1449 IDENTIFICATION THROUGH GENE EXPRESSION OF A TOXIC RESPONSE IN THE RAT FOR A COMPOUND THAT EXHIBITS OVERT TOXICITY IN HUMANS, BUT NOT IN RATS.

M. W. Porter, M. Elashoff, K. R. Johnson, A. L. Castle, M. S. Ott, H. W. Sun, C. G. Chang and D. L. Mendrick. *Toxicology, Gene Logic, Inc., Gaithersburg, MD.*

The classical measures of toxic response have been found to be insensitive to certain known hepatotoxicants, such as those that cause damage in humans, but whose effects are not detectable within the mammalian species used in pre-clinical trials. The implementation of a more sensitive measure of the toxic response to identify this type of compound is needed to assess accurately the potential toxicity of compounds in humans. For a variety of reasons, gene expression measurements from animal models may provide the means necessary to determine the eventual toxic response observed in humans. Tacrine is one such compound where an overt hepatotoxic response is observed in humans, but not in pre-clinical animal models. To identify the genes that characterize a response in the absence of overt hepatotoxicity, tacrine was administered to Sprague-Dawley male rats and liver tissue was isolated at various time points post-exposure. Using the Affymetrix RGU 34 GeneChip[®] set as a platform for measurement of gene expression levels, we have identified rat genes that are strong indicators of a toxic response for tacrine, but not the vehicle-treated controls. In addition, these genes have been previously shown to be good collective indicators of toxicity using samples extracted from rats treated

1,1 Styrene
1,1 Phenyl Ethyl Alcohol
Landseidel R. (USA)