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Clarifying carcinogenicity of ethylbenzene

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Abstract

Ethylbenzene has been evaluated for carcinogenic activity in Fischer rats and B6C3F1 mice exposed by inhalation [Chan et al 1998; Chan & NTP 1999] and in Sprague-Dawley rats after oral exposure [Maltoni et al 1985, 1997]. Bioassay findings are summarized below to expand on those not stated clearly or completely in Saghir et al [2010]. Overall in these three studies animals exposed to ethylbenzene had increased tumors in rats for kidneys, testes, head [including rare neuroesthesioepitheliomas], and total malignant tumors, whilst in mice tumors incidences were increased in the lung and liver [Huff, 2002]. Thus ethylbenzene was carcinogenic by two exposure routes to both sexes of two species of rodents, two strains of rats, and one strain of mice, causing collectively tumors in five different target organs and a composite of “total malignant” tumors.

Keywords

Cancer Bioassay; Carcinogenesis; Chemicals; Long-term Study; Occupational Cancer; Primary Prevention

Commentary

Saghir et al. [2010] insist that these carcinogenic responses in animals are not relevant to humans at environmental or occupational exposure levels, and hence mechanistically should not be considered a cancer risk. Also cited to discount these effects were ‘high doses’ and ‘cellular toxicity’ leading to or preconditioning the animals to cancer. Neither of these arguments has been shown germane to their thesis [e.g., Bucher, 2002; Hoel et al. 1988; Huff, 1992, 1993, 1995]. Over and over again cancer bioassay findings do not support any remotely consistent influence of toxicity -- irritation, inflammation, cellular degeneration, cellular turnover, cell proliferation -- and cancer [e.g., Farber 1995, 1996; Hoel et al. 1988; Huff 1991, 1992, 1995; Malnick 1992; Melnick & Huff 1993; Melnick et al. 1993, 1996, 1998; Tomatis 1993; Ward et al. 1993; Weinstein 1991, 1992, 1993]. As a counter example, among many, inhalation of tetranitromethane caused irritation of nasal passages and no tumors yet induced pulmonary carcinogenesis without any observed lung toxicity [Bucher & NTP 1990; Bucher et al. 1991]. These ‘anti-correlation’ cancers are not uncommon in NTP bioassays.

Regarding the authors’ “high dose only effects” claim, with incidences of 14 vs 20, 30, 38% for alveolar/bronchiolar adenoma/carcinoma of the lung in male mice, and 6 vs 10, 16, 42%

The authors declare that there are no conflicts of interest.

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for renal tubule adenomas/carcinomas in male rats, anyone claiming this is a “high-dose only” effect is either oblivious to the concept of dose-response or has a vested agenda. Significant dose response trends [$P < 0.01$] were evident for both these sites, and it is difficult to get much better linear dose-responses for animal tumor data. This is especially true given the uneven spread of exposures doses as chosen from shorter term studies [Chan 1992]; for the NTP studies: 0 vs 75, 250, or 750 ppm ethylbenzene by inhalation, 6 hours per day, 5 days per week, for 103 weeks [Chan et al 1998; Chan & NTP 1999].

Another posed argument is genetic toxicity and cancer. Again there is little convincing and dependable evidence that one can correlate either artificial “grouping” of genotoxic versus non-genotoxic chemicals for or against producing cancer in rodents or humans. Melnick et al [1996] evaluated this issue of non-genotoxic carcinogens and concluded with these interpretations “a) many chemicals considered to be nongenotoxic carcinogens actually possess certain genotoxic activities, and limiting evaluations of carcinogenicity to their nongenotoxic effects can be misleading; b) some nongenotoxic activities may cause oxidative DNA damage and thereby initiate carcinogenesis; c) although cell replication is involved in tumor development, cytotoxicity and mitogenesis do not reliably predict carcinogenesis; d) a threshold tumor response is not an inevitable result of a receptor-mediated mechanism. There are insufficient data on the chemicals reviewed here to justify treating their carcinogenic effects in animals as irrelevant for evaluating human risk.”

If ethylbenzene metabolism ‘saturates’ between 200 and 500 ppm [Saghir et al.2010], then, in organs where ethylbenzene is metabolized, one would not expect to see much increase in tumor incidence in the top exposure [750 ppm] compared to the mid level exposure [250 ppm], because both exposures are in the “saturation zone”. However, significant increases between these concentrations were obvious for liver, lung, and kidney. Also, the purported requirement for high levels of metabolism of ethylbenzene in human lung tissue to influence lung tumor risk may be false, because there is no reason why reactive metabolites formed in the liver of humans don't or couldn't distribute through blood to the lungs.

Additionally, several nonneoplastic lesions induced by ethylbenzene were dose-related: male and female rats with renal tubule hyperplasia and severities of nephropathy, male mice showing alveolar epithelial metaplasia, syncytial alteration of hepatocytes, hepatocyte necrosis, and thyroid gland follicular cell hyperplasia, and female mice exhibiting pituitary gland pars distalis hyperplasia, and thyroid gland follicular cell hyperplasia.

The statement “very low binding activity of human lung microsomes could be attributed to low enzymatic activity of the microsomal preparation due to autolysis of lung tissues from the time of death of the donors and harvesting and processing of tissues” [Saghir et al2010,pg 13] clearly indicates that their human data is totally useless. Because of polymorphisms in genes that code for metabolizing enzymes, use of a pooled human samples gives no information on variability or the range of activities among individuals. For these reasons alone this paper should have been better scrutinized, and perhaps could have been rejected unless explained satisfactorily.

It is amazing that even Dow Chemical Company would suggest in vitro binding to microsomal protein is a possible mechanism of mouse lung tumorigenesis. Based on the suggested mechanism of “increased toxicity to the mouse lung”, why then were there no adverse effects on survival or body weight in the NTP 2-year studies and no evidence of histopathologic injury to the lungs of mice exposed for 13 weeks to ethylbenzene at concentrations up to 1000 ppm? In fact, in these 13-week studies, “Chemically related histopathologic changes were not observed in any tissues of rats or mice” [Chan 1992]. The Dow paper [Saghir et al.2010] also

fails to address binding to DNA by the “electrophilic reactive metabolites” that bind to microsomal proteins.

Certain chemicals, mixtures of chemicals, exposure circumstances, life-styles and personal or cultural habits, occupations, viruses, living conditions, and physical agents have been causally associated with cancers in humans. Most of these varied exposures to chemicals however are not considered potentially carcinogenic, and the proportion of ‘agents’ eventually identified to cause cancer is projected to be relatively low [Fung et al.1996;Huff et al.1985]. However when a chemical convincingly causes cancers in animals, as does ethylbenzene, we should quickly pay serious attention rather than conjuring ways to discount these warnings of potential cancer in humans, especially workers exposed occupationally [Huff 2010a,b;Tomatis et al.1997, 2001]. IARC [2000] reviewed the available information on ethylbenzene and decided “There is sufficient evidence in experimental animals for the carcinogenicity of Ethylbenzene”, with an “Overall evaluation: Ethylbenzene is possibly carcinogenic to humans (Group 2B).”

Long-term carcinogenesis bioassays using experimental animals are the most predictive method for identifying likely human carcinogens. Since the 1960s, bioassays have proven a mainstay for identifying chemical carcinogens, establishing occupational exposure standards, and primary cancer prevention. The reasons, rationale, and validity are many [Huff,2010b]. Long-term bioassays are both predictive [prospective] and confirmatory [retrospective] for human carcinogens, and there has long been an agreeable association between carcinogenic outcomes from bioassays and human cancer hazards [Huff,1999;Tomatis,2000]. These correlations stem from accumulated evidence over the last 50 years during the modern era of experimental carcinogenesis [Tomatis & Huff 2002].

References

- Bucher JR, NTP. NTP Toxicology and Carcinogenesis Studies of Tetranitromethane (CAS No. 509-14-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). National Toxicology Program. Natl Toxicol Program Tech Rep Ser 1990 Mar;386:1–207. [PubMed: 12637973]
- Bucher JR, Huff JE, Jokinen MP, Haseman JK, Stedham M, Cholakakis JM. Inhalation of tetranitromethane causes nasal passage irritation and pulmonary carcinogenesis in rodents. *Cancer Lett* 1991 May 1;57 (2):95–101. [PubMed: 1851054]
- Bucher JR. The National Toxicology Program rodent bioassay: designs, interpretations, and scientific contributions. *Ann N Y Acad Sci* 2002 Dec;982:198–207. [PubMed: 12562638]
- Chan P. NTP technical report on the toxicity studies of Ethylbenzene (Cas No. 100-41-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). *Toxic Rep Ser* 1992 Mar;10:1–B7. [PubMed: 12209176]
- Chan PC, Haseman JK, Mahleri J, Aranyi C. Tumor induction in F344/N rats and B6C3F1 mice following inhalation exposure to ethylbenzene. *Toxicol Lett* 1998 Sep 30;99(1):23–32. [PubMed: 9801027]
- Chan P, NTP. NTP Toxicology and Carcinogenesis Studies of Ethylbenzene (CAS No. 100-41-4) in F344/N Rats and B6C3F1 Mice (Inhalation Studies). *Natl Toxicol Program Tech Rep Ser* 1999 Jan; 466:1–231. [PubMed: 12579207]
- Farber E. Cell proliferation as a major risk factor for cancer: a concept of doubtful validity. *Cancer Res* 1995 Sep 1;55(17):3759–62. [PubMed: 7641190]
- Farber E. Cell proliferation is not a major risk factor for cancer. *Mod Pathol* 1996 Jun;9(6):606. [PubMed: 8782195]
- Fung VA, Barrett JC, Huff J. The carcinogenesis bioassay in perspective: application in identifying human cancer hazards. *Environ Health Perspect* 1995 Jul-Aug;103(7-8):680–3. [PubMed: 7588478]
- Hoel DG, Haseman JK, Hogan MD, Huff J, McConnell EE. The impact of toxicity on carcinogenicity studies: implications for risk assessment. *Carcinogenesis* 1988 Nov;9(11):2045–52. [PubMed: 3052903]
- Huff J, McConnell EE, Haseman JK. On the proportion of positive results in carcinogenicity studies in animals. *Environ Mutagen* 1985;7(4):427–8. [PubMed: 4054069]

- Huff J. Chemical toxicity and chemical carcinogenesis Is there a causal connection? A comparative morphological evaluation of 1500 experiments. *IARC Sci Publ* 1992;116:437–75. [PubMed: 1428093]
- Huff J. Absence of morphologic correlation between chemical toxicity and chemical carcinogenesis. *Environ Health Perspect* 1993 Dec;101(Suppl 5):45–53. [PubMed: 8013424]
- Huff J. Mechanisms, chemical carcinogenesis, and risk assessment: cell proliferation and cancer. *Am J Ind Med* 1995 Feb;27(2):293–300. [PubMed: 7755018]
- Huff J. Long-term chemical carcinogenesis bioassays predict human cancer hazards. Issues, controversies, and uncertainties. *Ann N Y Acad Sci* 1999;895:56–79. [PubMed: 10676409]
- Huff J. Chemicals studied and evaluated in long-term carcinogenesis bioassays by both the Ramazzini Foundation and the National Toxicology Program: in tribute to Cesare Maltoni and David Rall. *Ann N Y Acad Sci* 2002 Dec;982:208–30. [PubMed: 12562639]
- Huff J. Occupational cancer and social inequities. *Eur J Public Health*. 2010a Apr 6; Epub ahead of print.
- Huff J. Predicting chemicals causing cancer in animals as human carcinogens. *Occup Environ Med*. 2010b in press.
- IARC. Ethylbenzene. *IARC Monogr Eval Carcinog Risks Hum* 2000;77:227–66. [PubMed: 11100403]
- Maltoni C, Conti B, Cotti G, Belpoggi F. Experimental studies on benzene carcinogenicity at the Bologna Institute of Oncology: Current results and ongoing research. *Am J Ind Med* 1985;7:415–446. [PubMed: 4003403]
- Maltoni C, Ciliberti A, Pinto C, Soffritti M, Belpoggi F, Menarini L. Results of long-term experimental carcinogenicity studies of the effects of gasoline, correlated fuels, and major gasoline aromatics on rats. *Ann N Y Acad Sci* 1997 Dec 26;837:15–52. [PubMed: 9472329]
- Melnick RL. Does chemically induced hepatocyte proliferation predict liver carcinogenesis? *FASEB J* 1992 Jun;6(9):2698–706. [PubMed: 1612294]
- Melnick RL, Huff J, Barrett JC, Maronpot RR, Lucier G, Portier CJ. Cell proliferation and chemical carcinogenesis: a symposium overview. *Mol Carcinog* 1993;7(3):135–8. [PubMed: 8489710]
- Melnick RL, Huff J. Liver carcinogenesis is not a predicted outcome of chemically induced hepatocyte proliferation. *Toxicol Ind Health* 1993 May-Jun;9(3):415–38. [PubMed: 8367884]
- Melnick RL, Kohn MC, Portier CJ. Implications for risk assessment of suggested nongenotoxic mechanisms of chemical carcinogenesis. *Environ Health Perspect* 1996 Mar;104(Suppl 1):123–34. [PubMed: 8722116]
- Melnick RL, Kohn MC, Dunnick JK, Leininger JR. Regenerative hyperplasia is not required for liver tumor induction in female B6C3F1 mice exposed to trihalomethanes. *Toxicol Appl Pharmacol* 1998 Jan;148(1):137–47. [PubMed: 9465273]
- Saghir SA, Zhang F, Rick DL, Kan L, Bus JS, Bartels MJ. In vitro metabolism and covalent binding of ethylbenzene to microsomal protein as possible a mechanism of ethylbenzene-induced mouse lung tumorigenesis. *Regul Toxicol Pharmacol* 2010 Jul-Aug;57(2-3):129–35. [PubMed: 20096743]
- Tomatis L. Cell proliferation and carcinogenesis: a brief history and current view based on an IARC workshop report. International Agency for Research on Cancer. *Environ Health Perspect* 1993 Dec;101(Suppl 5):149–51. [PubMed: 8013403]
- Tomatis L, Huff J, Hertz-Picciotto I, Sandler DP, Bucher J, Boffetta P, Axelson O, Blair A, Taylor J, Stayner L, Barrett JC. Avoided and avoidable risks of cancer. *Carcinogenesis* 1997 Jan;18(1):97–105. [PubMed: 9054595]
- Tomatis L. The identification of human carcinogens and primary prevention of cancer. *Mutat Res* 2000 Apr;462(2-3):407–21. [PubMed: 11523541]
- Tomatis L, Melnick RL, Haseman J, Barrett JC, Huff J. Alleged misconceptions' distort perceptions of environmental cancer risks. *FASEB J* 2001 Jan;15(1):195–203. [PubMed: 11149907]
- Tomatis, L.; Huff, J. Evolution of research on cancer etiology. In: Coleman, WB.; Tsongalis, GJ., editors. *The Molecular Basis of Human Cancer: Genomic Instability and Molecular Mutation in Neoplastic Transformation*. Vol. Chapter 9. Humana Press Inc.; Totowa, NJ: 2002. p. 189-201.
- Ward JM, Uno H, Kurata Y, Weghorst CM, Jang JJ. Cell proliferation not associated with carcinogenesis in rodents and humans. *Environ Health Perspect* 1993 Dec;101(Suppl 5):125–35. [PubMed: 8013399]

- Weinstein IB. Mitogenesis is only one factor in carcinogenesis. *Science* 1991 Jan 25;251(4992):387–8. [PubMed: 1989073]
- Weinstein IB. Toxicity, cell proliferation, and carcinogenesis. *Mol Carcinog* 1992;5(1):2–3. [PubMed: 1543538]
- Weinstein IB. Cell proliferation: concluding remarks. *Environ Health Perspect* 1993 Dec;101(Suppl 5): 159–161. [PubMed: 17539102]