HAZARD REVIEW

OF

ETHYLENEIMINE (EI)
(AZIRIDINE)

May 1973

Prepared by

Donald V. Lassiter, Ph.D.
Office of Research and Standards Development

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
National Center for Disease Control
National Institute for Occupational Safety and Health
Rockville, Maryland

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	PB87-210803

4. Title and Subtitle
Hazard Review of Ethyleneimine (EI) (Aziridine)

5. Report Date
May 1973

6.

7. Author(s)
Lassiter, D.V.

8. Performing Organization Rept. No.

9. Performing Organization Name and Address
NIOSH
4676 Columbia Parkway
Cincinnati, Ohio 45226

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.
(C)
(G)

12. Sponsoring Organization Name and Address
NIOSH
4676 Columbia Parkway
Cincinnati, Ohio 45226

13. Type of Report & Period Covered

14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)
The toxic hazards associated with exposure to ethyleneimine (EI) are discussed. Uses in textile treatment, adhesives and binders, rocket and jet fuels, chemosterilant chemicals, and chemotherapeutic agents are listed among the applications of EI. The hepatotoxic and neurotoxic effects of EI in rats and rabbits are noted, along with its mutagenicity in Drosophila, Neurospora, and plants and induction of chromosome aberrations in human and mouse cell cultures. Research findings are summarized for carcinogenicity tests of EI and related compounds in rats, including induction of injection site sarcomata, pancreatic tumors, mammary fibroadenoma, injection site fibroma, and renal carcinoma. The comparative carcinogenicity of EI and related compounds is discussed. Tumor induction is also described in mice injected subcutaneously with aziridine, a closely related derivative of EI. Also described is a study by the National Cancer Institute in which EI was used as a positive control compound. The author notes that the carcinogenicity of EI to humans depends upon the extrapolation of test results in mice and rats. Previous researchers have stated that, regardless of the EI derivative, the EI prosthetic moiety may be primarily responsible for the carcinogenicity of the molecule.

17. Document Analysis a. Descriptors

NIOSH-Publication NIOSH-Author Health-hazards Industrial-chemicals Employee-exposure Animal-studies Toxic-effects Mutagenicity Carcinogens Comparative-toxicology Ethyleneimine

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
AVAILABLE TO THE PUBLIC	UNCLASSIFIED	8
	20. Security Class (This Page) UNCLASSIFIED	22. Price

Hazard Review

of

Ethyleneimine (EI)

(Aziridine)

Ethyleneimine (EI) is an extremely reactive, small ring heterocyclic alkylating agent capable of undergoing both ring-opening and ring-preserving reactions. The dual-functionality and high degree of reactivity of EI are responsible for the synthesis of many derivatives of an increasing list of applications [1] including: textile treatment chemicals; adhesives and binders; rocket and jet fuels; chemosterilant chemicals; and chemotherapeutic agents to name a few.

Early interest in EI centered on its possible application as an antineoplastic agent. Because of the general type of cytotoxic activity associated with EI in this role, subsequent investigators were led to question its possible role as a chemical mutagen and carcinogen.

Because of its high reactivity EI is very irritating to the skin and mucous membranes. The renal toxicity of EI in the rat and rabbit has been described by Davies et al [2,3,4], and this investigator demonstrated that as little as 0.01 ml/kg body weight caused the death of rabbits from neurotoxic effects within a few hours. The toxicity

of EI has also been investigated by Walpole et al [5], who determined the LD(50) for mice and the maximum tolerated dose for rats.

The mutagenicity of EI has been demonstrated in Drosophila [6 through 10], Neurospora [11], and plants. [12 and 13] EI has also been discovered to cause chromosomal aberrations in human [14] and mouse [15] cell cultures.

The most definitive early investigations concerning the carcinogenic potential of EI were those of Walpole et al [5] published in 1954. Walpole et al conducted toxicity and carcinogenicity testing of EI and 20 related compounds, including 15 monofunctional aziridines. An important conclusion drawn from this study by the investigators was that the carcinogenic activity of the aziridines tested appeared "...to depend mainly upon the presence in their molecules of the ethyleneimine radical, acting, we suggest, as a prosthetic group by means of which they are able to combine with certain cell constituents. They can be regarded as the prototypes of a new range of carcinogens built up by introducing the ethyleneimine ring into organic chemical systems of diverse types." This conclusion was amply demonstrated in the study by the carcinogenic potential of many of the aziridines.

The study itself involved the subcutaneous injection (right flank) of 12 rats (6 male; 6 female) twice weekly with one of the test aziridines dissolved in sterilized arachis oil. The dose was

proportional to body weight and was near to that maximally tolerated. For EI, a total dose of 2 mg/100 g body weight in 2.0 ml of arachis oil was administered over a 67 day test period. Five male and one female developed sarcomata at the site of injection by termination of the 546-day experiment in an average time of 400 days from the initiation of dosing.

Vehicle control animals (10 male; 9 female) injected with arachis oil (total dose of 5 ml/100 g body weight) developed several types of tumors including: 1 injection site sarcomata (male); 1 carcinoma of the pancreas (male); 1 Islet cell adenoma of the pancreas (male); 1 mammary fibroadenoma (female); 1 injection site fibroma (female) and 1 carcinoma of the uterus. The one injection site sarcomata developed 568 days following the initiation of the 67 day dosing protocol and the maximum time for development of all other tumors was 590 days.

To determine the effect of the arachis oil a second study was conducted which was similar to the first except that EI was dissolved in water as the vehicle. A total dose of 1.2 mg/100 g body weight in 1.2 ml water was administered to 6 male rats and 1.0 mg/100 g body weight in 1.0 ml water to 6 females according to the injection protocol of the initial experiment. In this series two females developed injection site sarcomata and one male developed a transitional cell carcinoma in the kidney. The investigators considered their results to leave little doubt that the injection site

sarcomata were attributable to the direct action of the ethyleneimine test compounds themselves. Of the 16 aziridine compounds tested, ethyleneimine exhibited the weakest carcinogenicity, except for three ethyleneiminosulphonyl alkanes. This finding demonstrates that monofunctional ethyleneimine derivatives possess greater carcinogenic potential than the parent compound itself. In fact, these investigators also reported the results of tests using the polyfunctional ethyleneimine derivative, triethylenemelamine (TEM), and discovered it to possess an even greater carcinogenic effect than any of the monofunctional ethyleneimines. However, these investigators conclude that EI, itself, may be the ultimate carcinogen, regardless of the derivative in question; the greater carcinogenic activity of electrically neutral mono- and poly-derivatives being directly attributable to their facility for cellular diffusion.

A closely-related ethyleneimine derivative, aziridine ethanol (beta-hydroxy-1-ethylaziridine), was discovered by Van Duuren et al, [16] in a study sponsored by the National Cancer Institute, to induce injection site sarcomas in 10/30 mice injected (s.c.) once weekly for 74 weeks with 0.3 mg/0.05 ml tricaprylin (vehicle). The sarcomas included: 4 fibrosarcomas; 1 adenosarcoma; and 5 lymphosarcomas. There were no tumors discovered in the vehicle control animals injected (s.c.) once weekly with 0.05 ml of tricaprylin.

In 1969, a study sponsored by the National Cancer Institute concerning the bioassay of industrial chemicals for tumorigenicity was published by Innes et al. [17] In this study EI was used as one of the positive control compounds. This compound was administered to 72 mice (36 males; 36 females) of two strains [(C57BL/6 X C3H/Anf)F_2 and (C57BL/6 X AKR)F_1] via the stomach tube (7-28 days of age) in a daily concentration of 4.64 mg/kg body weight in 0.5 percent gelatin. After the animals were 28 days old EI was included in their diet at a concentration of 13 ppm for approximately 77 weeks. All doses were considered to be those which could be maximally tolerated. By the termination of the study 58 of the 72 exposed mice developed tumors: 24 males and 13 females had hepatomas; 27 males and 25 females had pulmonary tumors and 2 females developed lymphomas. When these results were compared to the results of negative control mice, with the exception of the 2 lymphomas, the increased tumor yield was significant at the 0.01 level. The investigators commented on their classification of liver tumors as "hepatomas", stating:

> "...the term 'hepatoma' as used in this manuscript should not be considered as implying that these tumors are benign. Indeed, it seems more reasonable to conclude that the great majority had malignant potentiality."

The investigators concluded by stating that the dose received by the mice was far above that likely to be received by humans but that there

was no way to predict whether man would be more or less susceptible to tumor induction by the compound.

The case for the carcinogenicity of EI, then, rests on the possible extrapolation to humans of the findings in two separate, controlled animal studies, one involving rats [5] and the other mice. [17] This position is compatible with that of NIOSH concerning the prior demonstration of carcinogenicity in at least two animal species. While some studies have documented the mutagenicity of EI [6 through 13] and its aberrational effect on chromosomes in animal and human cell cultures [14 and 15], still others have surveyed the carcinogenicity of EI derivatives [5 and 16] and discovered polyfunctional derivatives to be much more carcinogenic than monofunctional ones. [5] In addition, it was considered by Walpole et al [5] that regardless of the EI derivative, the EI prosthetic moiety may be primarily responsible for the carcinogenicity of the molecule.

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