HAP Task Force

2001 L Street, N.W., Suite 506A, Washington, D.C. 20036

February 27, 1998

Document Control Office (7407)
Office of Pollution Prevention and Toxics
Room G-099
401 M Street, S.W.
U.S. Environmental Protection Agency
Washington, DC 20460

Dear Mr. Auer:

Please find enclosed the HAP Task Force's revised detailed evaluation of the need to conduct a multigeneration study for ethylene dichloride (EDC) based on an examination of the relevant published literature. Since our meeting on January 12, 1998 we have obtained and evaluated an additional reproductive study by Alumot et al. entitled "Tolerance and Acceptable Daily Intake of Chlorinated Fumigants in the Rat Diet" in which EDC was administered to rats for two years (attached). Growth fertility and reproductive and biochemical tests were used as criteria to establish a no-effect level.

We would appreciate your review of our evaluation of these data in assessing the potential human reproductive effects of EDC and look forward to further discussions and completing protocol outlines for the other endpoints discussed at our meeting for EDC.

Our willingness to pursue an ECA for EDC is without prejudice to the Task Force's position as to the need for the proposed testing or EPA's statutory authority to require it, which will be addressed in our comments on the proposed test rule.

If you have any questions please contact me at 202-775-0232.

Sincerely,

Peter Voytek, Ph.D.

cc Charles Auer
Rich Leukroth
Annie Jarabek

DO A 116817
CONFIDENTIAL

DRAFT

Detailed Evaluation of the Reproductive Toxicity Testing Requirement for Ethylene Dichloride (EDC) Under TSCA

A. Introduction

Background: On June 26, 1996, the USEPA proposed a test rule under Section 4(a) of the Toxic Substances Control Act (TSCA) which would require manufacturers and processors of 21 hazardous air pollutants to test these compounds for specific health effects. As an alternative to conducting toxicological studies, USEPA solicited pharmacokinetic (PK) proposals, which could allow route-to-route extrapolation of existing data to fill the data gaps identified in the test rule. These proposals may lead to the development of enforceable consent agreements (ECAs). In response to this request, a PK proposal was prepared and submitted to USEPA by the HAP Task Force for ethylene dichloride (EDC) in November of 1996. In June of 1997, USEPA released preliminary technical analyzes of the proposed pharmacokinetic strategies for EDC. A public meeting was held on January 12, 1998 to discuss the development of an ECA for EDC. One of the items of discussion pertained to the reproductive toxicity testing requirement. A case was presented by ChemRisk on behalf of the HAP Task Force that the existing studies for EDC provide a "weight-of-evidence" that demonstrates reproductive endpoints are not of concern. EPA agreed to consider alternative approaches for addressing reproductive toxicity testing needs for EDC. This document serves as a proposal for addressing these needs under an ECA.

Toxicity testing requirements can be modified when warranted by scientific merit and judgement. Although there is considerable data for reproductive endpoints for EDC, it is recognized that no single study fulfills all current TSCA testing requirements. There are two reasons this may occur. First, the number of toxicological endpoints of potential interest increases as our understanding of chemical interaction with biological systems increases. Second, the sensitivity of analytical methods used to detect effects for existing endpoints is also likely to improve.

At issue is the following question, *"How does one update existing toxicological information to provide adequate data for assessing reproductive risks?"*. In general, two approaches are possible. Under the first approach, a new study is conducted that evaluates all endpoints of interest by itself to satisfy TSCA testing requirements (*i.e.*, existing information are ignored). Under the second approach, all available information are used to define what is known and what is not known regarding specific endpoints. Studies are then designed and implemented as supplemental studies to specifically address newly identified endpoints. The HAP Task Force considers there to be sufficient merit in pursuing EDC through the latter approach. There exists an excellent database of studies regarding reproductive endpoints for EDC. However, data gaps remain for a few endpoints that have recently come into focus.

B. Existing Studies for EDC

Three reproductive studies are currently available for EDC, two of which were presented to the Agency at the January 12th meeting (Lane et al. 1982; Rao et al. 1980). Since the meeting, a third reproductive study was identified for EDC (Alumot et al. 1976). All three reproductive studies are described briefly below.

1. *Lane et al. (1982)* - In a multigeneration study, groups of 10 male and 30 female ICR Swiss mice were exposed to 0, 5, 15, or 50 mg/kg-day EDC via the drinking water. The F0 generation was exposed for 5 weeks prior to mating, whereas the F1 generation was exposed to 11 weeks prior to mating. No treatment-related effects on fertility, gestation, terata, pup weight gain, pup survival, or dominant lethal mutations were observed. This study identifies a NOAEL of 50 mg/kg-day for the reproductive effects of EDC.

2. *Rao et al. (1980, also cited as Murray et al. 1980, Schlacter et al. 1979, and Shell Oil 1979)* - In a single generation study, groups of 20-30 male and 20-30 female Sprague-Dawley rats were exposed via inhalation to 0, 25, 75, or 150 ppm EDC for 6 hours/day beginning 60 days prior to mating, and continuing through gestation (F1a and F1b). No treatment related effects were observed on fertility index, pup survival, gestation length, sex ratio, or organ weights in pups from either the F1a or F1b litters. This study identifies a NOAEL of 150 ppm for the reproductive effects of EDC.

3. *Alumot et al. (1976)* - In a single generation study, groups of 18 male and 18 female rats (species not specied) were exposed to mash fumigated with EDC. Residue levels measured 250 and 500 ppm, corresponding to doses of approximately 13 and 25 mg/kg-day, respectively. After 6-weeks on the test diet, females were mated with untreated males. Thereafter, at 2-monthly intervals, 45 treated males were mated with treated females for a total of 24 months. Following mating, females were weighed twice weekly. Litter size and weight were recorded at parturition and at 10 days. Liver fat content and serum biochemistry analyzes were performed on parental animals at the end of the treatment period, and did not reveal significant effects. No treatment related effects were reported on reproductive success in animals followed for up to 5 sequential pregnancies during a two-year exposure period. As such, this study provides excellent information regarding the potential for reproductive effects in aging animals (an important endpoint which is <u>not</u> specifically addressed in the TSCA testing requirements). This study identifies a NOAEL of 25 mg/kg-day for the reproductive effects of EDC.

Although neither the study of Rao et al. 1980 nor the study of Alumot et al. (1976) satisfy all current TSCA testing requirements, these studies do provide useful information regarding the potential for reproductive effects of EDC. Because a second generation was evaluated in the Lane et al. (1982) study, this study warrants additional consideration.

The study design for the Lane et al. (1982), which was approved by EPA, does evaluate a second generation. The study protocol is provided in Figure 1. A detailed comparison of this study to

current TSCA testing requirements is provided in Table 1. Based on this comparison, the attributes of the Lane et al. (1982) study can be categorized in one of three ways: (1) requirements that are met; (2) requirements that are exceeded; and (3) requirements that are not met. Examples of each category are summarized below.

Points where requirements are met by Lane et al. (1982)

- Adequacy of test species: Swiss mice
- Adequate number of dose groups: Four (including control)
- Adequate study design: Two-generations
- Adequate evaluation of the following endpoints: gross necropsy, body weight, fertility index, gestation/lactation index.

Points where requirements are exceeded by Lane et al. (1982)

- Number of pregnant animals/dose group: 20 required, 30 tested
- Number of F1 and F2 matings: 1 F1 required, 3 tested; 1 F2 required, 2 tested
- Dominant lethal mutations: Not required under TSCA

Points where requirements are not met by Lane et al. (1982)

- Lack of evaluation of the following endpoints: organ weights, histopathology, estrous cyclicity, sperm count/morphology, age at sexual maturation. Each of these endpoints is discussed in more detail in the next section.

C. Discussion of Potential Limitations of Lane et al. (1982)

Several were identified above for the Lane et al. (1982) study. These are discussed below.

- *Organ Weights* - Organ weights were not measured in F0, F1, or F2 animals by Lane et al. (1982). However, this endpoint is of questionable significance, since changes in organ weight usually do not constitute an adverse effect. Furthermore, since organ weights for reproductive tissues were in several other studies without remarkable findings (Cheever et al. 1990; Daniel et al. 1994; Rao et al. 1980; NTP, 1991), this does not constitute a serious limitation in the study design.

- *Histopathology* - Histopathological examinations of F0, F1, or F2 animals were not conducted by Lane et al. (1982). Regarding F0 animals, several other studies have evaluated histopathology of reproductive tissues following oral and inhalation exposures (Rao et al. 1980; NTP, 1991; Daniel et al. 1994; Cheever et al. 1990), none of which report any histopathological in reproductive tissues. In addition, histopathological changes of the liver, kidney, and any other target organs noted during gross examination have been evaluated in F1 rat pups (Rao et al. 1980), again without notable effects. However, regarding F1/F2 animals, this is no longer required since,

> "EPA dropped the requirement of histopathology of developmental anomalies observed macroscopically in F1 and F2 weanlings." (Federal Register: August 15, 1997).

For this reason, the absence of histopathology does not constitute a significant limitation in this study.

- *Sperm Count, Motility, Morphology* - Sperm structure and function were not evaluated by Lane et al. (1982). Although these were not part of the multigeneration protocol, the HAP Task Force feels that there is merit to considering effects on gametogenesis triggered as endpoints, as was done for primordial follicles under the TSCA reproductive test guidelines. In addition, the HAP Task Force would like EPA to consider the dominant lethal mutation assay (Lane et al. 1982) as a more relevant endpoint for EDC, since it is recognized as a DNA reactive chemical.

- *Estrous Cyclicity* - Estrous cyclicity was not evaluated by Lane et al. (1982). Halogenated organic compounds that are known to affect the estrous cycle such as DDT, methoxychlor, PCBs are generally believed to produce their effects via interaction with the estrogen receptor. Given the chemical structure of EDC, it is very unlikely that it exerts any estrogenic (or other hormonal) activity.

- *Age at Sexual Maturation* - The age at sexual maturity (day of vaginal opening/preputial separation) was not assessed by Lane et al. (1982). Like estrous cyclicity, halogenated organic chemicals which are known to speed or delay the onset of sexual maturity (including DDT, methoxychlor, polychlorinated biphenyls, polybrominated biphenyls) are generally believed to produce their effects via interaction with the estrogen receptor. Given the chemical structure of EDC, it is very unlikely that it exerts any estrogenic (or other hormonal) activity.

D. Recommendations

In summary, the deficiencies noted for the Lane et al. (1982) study can be characterized as minor. Two endpoints (organ weight, histopathology) are readily addressed by other studies, or are no longer applicable. The remaining three endpoints (sperm morphology, estrous cyclicity, age at sexual maturation) are certainly recognized as important endpoints, particularly with the recent attention paid to endocrine active compounds. However, these endpoints are more appropriately cast as those aimed at understanding the mechanism by which a chemical affects reproductive success. Because the weight-of-evidence (Alumot et al. 1976; Rao et al. 1980; Lane et al. 1982) clearly indicates that EDC has no effect on reproductive success, the importance of these mechanistic endpoints is greatly reduced. Furthermore, the likelihood of obtaining a positive response for these endpoints (if assayed) is small. The HAPs Task Force maintains that additional testing for the reproductive effects of EDC is not warranted. In light of the recently identified reference (Alumot et al. 1976), which further

supports previously presented arguments, the HAPs Task Force requests that EPA reconsider the reproductive testing requirements for EDC.

E. References

Lane RW, Riddle BL, Borzelleca JF. 1982. Effects of 1,2-dichloroethane and 1,1,1-trichloroethane in drinking water on reproduction and development in mice. Toxicol Appl Pharmacol 63:409-421.

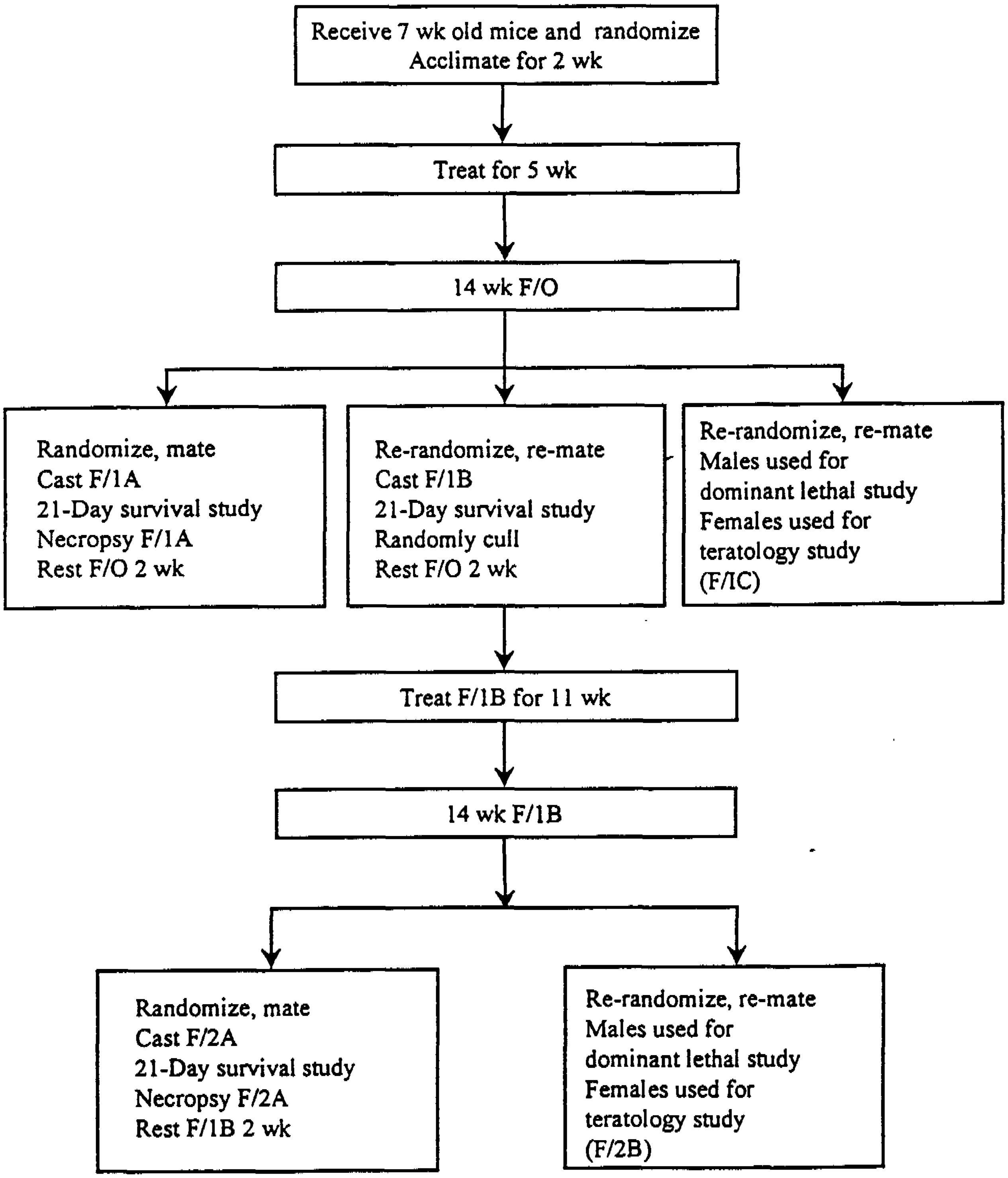
Rao KS, Murray JS, Deacon MM, et al. 1980. Teratogenicity and reproduction studies in animals inhaling ethylene dichloride. In: Ames, B, Infante P, Reitz R, eds. Ethylene dichloride: A potential health risk? Banbury report No. 5. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 149-166.

Alumot E, Nachtomi E, Mandel E, et al. 1976. Tolerance and acceptable daily intake of chlorinated fumigants in the rat diet. Food Cosmet Toxicol 14:105-110.

Table 1.
Comparison of Lane et al. (1982) to TSCA Guidelines for Reproductive Toxicity

Parameter	Recommended	Lane et al. 1982
Species	Rat (other mammal)	Mouse
Strain	Common laboratory	Swiss
Age	Young adult	Adequate
Number of animals	> 20 pregnant females	10M 30F/group
Doses	> 4 (including control)	0, 5, 15, 50 mg/kg-day
Route	Inhalation	Oral (drinking water)
Exposure Frequency	7 d/wk	7 d/wk
Design	Multigenerational; exposure > 10 wks prior to mating	Multigenerational; 5 wks prior to F1 mating, 11 wks prior to F2
Number of F1 mating	1	3
Number of F2 mating	1	2
Mating	Random	Random/Rerandomized
Control	Untreated/vehicle	Untreated/vehicle
Observations (adult)	Daily/weekly	Weekly
Observations (litter)	PND 0, 4, 7, 14, 21	PND 0, 4, 7, 14, 21
P	Estrous cyclicity	No
	Gross necropsy	Yes
	Histopathology	No
P&F1	Sperm count/motility/morphology	No
	Organ weights	No
F1&F2	Gross necropsy	Yes
	Body weight measurement (ld 4, 7, 14, 21)	Yes
	Fertility index	Yes
	Gestation/Lactation index	Yes
	Age at sexual maturation	No
	Organ weights	No
	Other	Dominant lethal mutation
Data reporting	Tabular	Adequate
Evaluation	Statistics described	Adequate

FIGURE 1
MULTIGENERATION DESIGN STUDY FOR EDC (Lane et al. 1982)


Receive 7 wk old mice and randomize
Acclimate for 2 wk
Treat for 5 wk
14 wk F/O
Randomize, mate
Cast F/1A
21-Day survival study
Necropsy F/1A
Rest F/O 2 wk
Re-randomize, re-mate
Cast F/1B
21-Day survival study
Randomly cull
Rest F/O 2 wk
Re-randomize, re-mate
Males used for
dominant lethal study
Females used for
teratology study
(F/IC)
Treat F/1B for 11 wk
14 wk F/1B
Randomize, mate
Cast F/2A
21-Day survival study
Necropsy F/2A
Rest F/1B 2 wk
Re-randomize, re-mate
Males used for
dominant lethal study
Females used for
teratology study
(F/2B)

DO A 116824
CONFIDENTIAL