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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDA

6(A)(2) DATA

SUBJECT: ID# 282892. Glutaraldehyde, technical. Evaluation of draft data for 2 year chronic feeding (drinking water) study in rat submitted as adverse effect data.

Tox. Chem No.: 468
Shaughnessey No.: ~~043601~~ 043901
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CONCLUSIONS:

A statistically significant increase in the incidence of large granular lymphocyte (LGL) leukemia was observed in female Fischer F344 rats treated for 2 years with glutaraldehyde at 50, 250 and 1000 ppm, administered *ad libitum* in drinking water. The data submitted in draft form meets the following flagging criteria for adverse effects as outlined in CFR 158.34, Reporting Code 1: increase of neoplasms in male or female animals which increases with dose or a statistically significant ($p < 0.05$) incidence of any type of neoplasm in any test group (male or female animals at any dose level) compared to concurrent control animals of the same sex.

At this time TB-I does not have sufficient information to determine potential carcinogenic risks of glutaraldehyde from the

results of this study. LGL leukemia is a spontaneously occurring neoplasm in F344 rats whose incidence is variable and can be quite high (20-25% and as high as 50%; males slightly higher than females). There is not enough information provided in the draft tables to determine whether the increases seen in treated females reflect the natural variability of spontaneous incidence of LGL leukemia in F344 rats or actual effects of glutaraldehyde to modulate tumor formation. TB-I defers this judgement pending receipt of the complete, final study including the following information: 1) individual animal data for all parameters, particularly needed to determine actual numbers of females affected by LGL leukemia, in addition to incidence of specific organ involvement, and to correlate mortality data with incidence of LGL leukemia; 2) historical control data for F344 rats, by study, from the Union Carbide laboratory for 2-3 years prior to and during, if possible, the study under consideration here and 3) final statistical analysis and Quality Assurance Statement.

ACTION REQUESTED:

Union Carbide submitted data taken from a 2 yr rat chronic toxicity/oncogenicity study (drinking water, study no. 54-43 performed at Union Carbide toxicology laboratories; MRID # 420476-01) under FIFRA 6(a)(2) requirement for notification of adverse effects. The chronic toxicity/oncogenicity study data submitted for consideration is in draft form and a data evaluation report will not be prepared at this time. The purpose of this brief review is to determine whether the findings in this study warrant 6(a)(2) classification and indicate a potential cancer risk for glutaraldehyde.

DISCUSSION:

Study Protocol: Fischer F344 rats were given drinking water containing 50, 250 and 1000 ppm glutaraldehyde (100 males and 100 females per control and treatment groups) for 104 weeks. Drinking water was the route of choice for administration of glutaraldehyde based on difficulties encountered with gavage and dietary administration. Unpalatability limited the dose of glutaraldehyde that could be administered in drinking water to 1000 ppm, and a reduction in water consumption was observed in mid and high dose males and females compared to controls. Animals were weighed and food and water consumption monitored throughout the study. Clinical chemistry, hematology, urinalysis, organ weight and histopathology parameters were analyzed.

Study Findings: The study authors' summary and conclusions as submitted to the EPA along with the draft data tables are appended to this memo. The most significant finding of this study was an increased incidence of large granular cell lymphocytic leukemia (LGL) in female rats. The increases were noted upon light

microscopic examination of liver and spleen. Table 1 below presents the incidence of this neoplasm for each group:

TABLE 1: INCIDENCE OF LGL LEUKEMIA IN CONTROL AND TREATED F344 RATS

TISSUE	SEX	0 PPM	50 PPM	250 PPM	1000 PPM
Spleen	M	43/100	51/100*	40/100*	46/100**
	F	24/100	41/100*	41/100*	53/100**
Liver	M	37/100	48/100*	39/100*	45/100**
	F	23/100	40/100*	40/100*	52/100**

* $p < 0.05$
** $p < 0.01$

The spontaneous incidence of LGL leukemia in spleen and liver of males was quite high in controls and all treatment groups (40-51%) and no significant increases were observed among treated male animals. Females also had relatively high incidence of LGL leukemia among control animals (23-24%) in liver and spleen but showed statistically significant increases at all treatment levels. Increases were identical at low and mid dose (41-42% incidence; 70% increase over controls) and were highest at high dose (52-53% incidence; 120% increase over controls) but did not exceed the incidences observed among males. The severity of the neoplasia as determined by microscopic evaluation increased slightly at high dose relative to controls.

Clinical correlations for LGL leukemia were few but included an increase in large monocytes in blood at Week 104. The increases were not, however, correlated with increased incidence of LGL. No other remarkable findings for hematology or clinical chemistry were observed that correlated with LGL leukemia. Necropsy observations included a small increased incidence of increased spleen size in females at mid and high dose (6/62 in controls, 10/52 and 11/56, mid and high dose, respectively). Among males that died during the study, LGL leukemia did not cause increased mortality among treated males (approximately half of the mortalities in each control and treatment group were caused by LGL). Among females, mortality data is presented below in Table 2:

TABLE 2: FEMALE MORTALITY IN CONTROL AND TREATED F344 RATS

DOSE GROUP:	0 PPM	50 PPM	100 PPM	1000 PPM
# ANIMALS EXAMINED (DEATHS DURING STUDY)	19	35	29	26
CAUSE OF DEATH: LGL				
# ANIMALS	11	18	18	20
% OF TOTAL DEATHS	58	51	62	77
DEATHS DUE TO LGL/ALL LGL ANIMALS (%)	46	44	53	38

Mortality among treated females was somewhat higher than controls but the increase was not dose-related and was highest at the low dose. The % of total deaths caused by LGL leukemia increased at high dose, but the increase was not statistically significant. Dates and causes of death for individual animals were not provided in the draft, but most of the deaths occurred after 52 Weeks. The percentage of control animals that died of LGL leukemia/total animals with LGL leukemia during the study was approximately the same as for treated animals; therefore glutaraldehyde did not appear to affect mortality rats with the tumor. The severity of LGL leukemia increased slightly with treatment as determined by histological examination of liver and spleen.

Other effects not related to LGL leukemia included decreased urine output and increased osmolality at mid and high dose in both males and females. This was probably due to the decreased water consumption rather than compound toxicity. Microscopic inspection of kidney revealed a dose-related increase in the incidence of renal tubule pigmentation at 104 Weeks; the increase was statistically significant at mid dose in females and at high dose in both males and females.

Decreased body weight and body weight gain was observed in both males and females at high dose and at mid dose in males only. Decreased body weight and weight gain were evident by Week 1 in males at both mid and high dose: body weights averaged approximately 2-4% lower at mid dose and 5-10% lower at high dose. Body weights of females decreased by Week 6-7 in females at high dose by 2 - 4% initially and by 6-8% towards the end of the study. Females consumed approximately 30% more compound per kg body weight than males.

Discussion: Large granular cell leukemia is one of several common spontaneous neoplasms occurring in older Fischer F344 rats. Spontaneous incidence varies somewhat from study to study but is usually around 20-25%, is slightly higher for males than females and can be as high as 50%. This neoplasm is rare in young animals and usually is observed in rats over 20 months of age.

The reason for the differences in control group tumor incidences between male and female rats is not clear. The study authors suggest that glutaraldehyde may modulate tumor formation in female F344 rats, based upon statistically significant increases of LGL leukemia in liver and spleen at all doses. Another possible explanation is that control females had a lower incidence than treated females due to normal variation in spontaneous incidence. This is a possibility since the incidence in treated females did not significantly exceed that of males, did not show a strong dose-response correlation and did not cause a significant increase in mortality. Since spontaneous incidence of LGL leukemia can be quite high, individual animal data and F344 rat historical control

data from Union Carbide's toxicology labs are needed to help evaluate the results of this study.

Based upon the data submitted in draft form, the study meets the flagging criteria for adverse effects outlined in the Conclusions section of this memo. The study data presented here indicates a possible treatment-related increased incidence of an already common spontaneous neoplasm in Fischer F-344 rats in females only. The final study report and historical controls as discussed under Conclusions and data from future chronic feeding/oncogenicity studies in other species will be needed to determine the potential cancer risks, if any, presented to humans by glutaraldehyde.

UNION CARBIDE

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Date: October 2, 1991

To: Mrs. Joan E. Young
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SUBJECT: GLUTARALDEHYDE

The information on glutaraldehyde [CASRN 111-30-8] given below is supplied to you as representing that which may meet the provisions of certain Regulations. It concerns the preliminary findings, as yet not subjected to Quality Assurance, from a combined chronic toxicity/oncogenicity study in rats given glutaraldehyde in the drinking water.

CURRENT STUDY

Study Design

The study was conducted to comply with the following regulations and guidelines: EPA Pesticide Assessment Guidelines (Subdivision F, Section 83-5, November, 1985). OECD Guidelines for Testing of Chemicals, No. 453, May 12, 1981, and Good Laboratory Practice Regulations, FIFRA, 40CFR Part 160.

Groups of Fischer 344 rats, each containing 100 males and 100 females, were given drinking water containing various concentrations of glutaraldehyde continuously for at least 104 weeks; a control group received drinking water not containing glutaraldehyde. The various glutaraldehyde concentrations in drinking water, and the corresponding daily glutaraldehyde consumptions averaged over the study period, are given in the Table below:

Glutaraldehyde (ppm in water)	Group Size	Average Daily Glutaraldehyde ± SD (mg/kg/day)	
		Males	Females
0.0 (Controls)	100M + 100F	0	0
50.0	100M + 100F	3.6 ± 0.76	5.5 ± 0.90
250.0	100M + 100F	17.1 ± 3.29	25.1 ± 4.17
1000.0	100M + 100F	63.9 ± 10.11	85.9 ± 11.51

Ten males and ten females were sacrificed at 12 months and 18 months into the study (interim sacrifices). Survivors were sacrificed at the end of the 2-year period. The following monitors for toxicity were conducted:

1. Daily observations for overt signs of toxic and/or pharmacological effects.
2. Weekly detailed clinical observations.
3. Body weights were measured before the start of dosing, and at weekly intervals for the first 13 weeks and alternate weeks thereafter.
4. Food and water consumption were measured at weekly intervals for the first 13 weeks and every other week thereafter.
5. Blood was collected at 3, 6, 12, 18 and 24 months (20 animals/sex/group) for hematology and clinical chemistry.
6. Urine was collected at 3, 6, 12, 18 and 24 months (10 animals/sex/group) for analysis of chemical and physical characteristics.
7. At the designated sacrifice time, animals were subjected to necropsy to examine for signs of gross pathology. Various organs were removed for weighing, and a large number of tissues and organs processed for examination by light microscopy. Animals that died or were sacrificed in a moribund condition were subjected to necropsy examination, and tissues and organs removed for histological examination.

Major Findings

1. Mortalities were as shown in appended Table 1.
2. Body weights and body weight gains were decreased in the 250 and 1000 ppm groups of males and 1000 ppm in females (Tables 5, 6, 7 and 8). Body weight or body weight gain were not affected for animals in the 50 ppm treatment group.
3. Water consumption was decreased in a dosage-related manner for males and females of the 250 and 1000 ppm groups from the start of the study, and sporadically at 50 ppm (Tables 9 and 10).
4. Food consumption was decreased intermittantly, though statistically significantly, in the 250 and 1000 ppm groups of both sexes. Food consumption was not affected for animals in the 50 ppm treatment group (Tables 11 and 12).
5. Urine volumes were decreased and urine osmolality increased in a dosage-related manner, being statistically significant at 250 and 1000 ppm for both males and females at week 12 (Tables 19 and 20), week 25 (Tables 25 and 26), and week 51 (Tables 31 and 32). At week 77, urine volumes and osmolality were only statistically significantly different at 1000 ppm (Tables 37 and 38). At week 104, decreased urine volume was only statistically significantly reduced at 1000 ppm in males, and osmolality increased significantly at 1000 ppm in females and 250 and 1000 ppm in males (Tables 43 and 44).

6. Kidney weight relative to body weight was statistically signficantly increased in males and females at 1000 ppm at the 12 and 18 months sacrifice, and absolute kidney weight was statistically significantly increased in females and decreased in males at the final sacrifice.

7. Histopathological findings of note were as follows with respect to large granular cell lymphocytic leukemia (abstracted from Tables 65, 66, 67, 70, 73, 74, 77, 78, 81 and 82):

Tissue	Sex	Incidence of LGL for Various Groups[a]			
		0 ppm	50 ppm	250 ppm	1000 ppm
Spleen	M	43/100	51/100	40/100	46/100
	F	24/100	41/100[b]	41/100[b]	53/100[c]
Liver	M	37/100	48/100	39/100	45/100
	F	23/100	40/100[b]	40/100[b]	52/100[c]

[a]LGL = Large granular cell lymphocytic leukemia.
Incidence given as [No. with LGL]/[Total number examined].
[b]$p<0.05$ compared to 0.0 ppm
[c]$p<0.01$ compared to 0.0 ppm

Thus, the incidence of large granular cell lymphocytic leukemia was statistically significantly increased on light microscopic examination of the liver and spleen of females at all concentrations of glutaraldehyde in drinking water. Data were statistically analyzed by the following approaches:

1. Fischer's exact
2. Fatal tumor (life-table) analysis)
3. Incidental tumor analysis
4. Cochran-Armitage linear trend test

The pattern of the response indicates that the increased incidence of large granular cell lymphocytic leukemia in females is unlikely to represent a direct chemical carcinogenic effect, but probably due to a modulating effect on this spontaneously occurring tumor in Fischer 344 rat. In view of the changing and variable incidence of large granular lymphocytic leukemia in the Fischer 344 rats, the results are currently being further analyzed along the following lines:

1. Farrar-Crump analysis for random tumor occurrence.
2. Comparison of the incidence of leukemia in the present study control animals with those from another chronic bioassay in the Fischer 344 rat currently reaching conclusion.
3. Analysis of the incidence of large granular cell lymphocytic leukemia with alterations in individual food and water consumption data.

The results supplied in the accompanying Tables have not yet been subjected to Quality Assurance, and should be considered as "Draft".

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