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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

002451

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: George LaRocca, PM #15
Insecticide Branch/RD (TS-767)

THRU: R. Bruce Jaeger, Section Head *RBJ 1/27/83*
Review Section #1
Toxicology Branch/HED (TS-769)

SUBJECT: Tetrachlorvinphos - toxicity to mice.
CASWELL # 217A

Background:

In a Shell 2-year feeding/oncogenicity study conducted by Hazelton Labs., Vienna, Va. tumors of the livers were seen in mice. Shell Chemical Company's position is that a dosage(s) greater than the NiD overwhelmed the normal adaptive and physiologic responses of the animals, and thus resulted in the liver hypertrophy. A short-term 14-day feeding study in male mice has been submitted to support that position.

Conclusion:

1. Data submitted do not alter previous considerations with regard to the liver effects elicited by high dietary doses of tetrachlorvinphos to male mice.
2. Toxicology Branch considers the data CORE: Supplementary.

Review:

Toxicity studies with SD 8447 (Gardona): Biochemical and morphological studies of the effects of short-term dietary exposure on mouse liver.

Experiment #1558 and 1729 reported by Tunstall Labs.
Report TLGR 80.125, dated 7/7/81; Acc. No. 246800, dated 2/12/82.

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Material Tested: SD 8447 (Z isomer)

Batch Number: F770384/S

Purity: 98.4% Gardona, Rabon, tetrachlorvinphos

Test Animal: CD1 male Charles River mice

Methods: Male mice, 3 weeks old were maintained as per SPF conditions. At 6 weeks, individual caging and feeding of respective diets (LAD1 & LAD2) took place.

The study was composed of 2 experiments, as follows:

#1. 10 or 14 males age 10 weeks were randomly assigned to each of seven groups, including control (diet LAD2), positive control with 500 ppm phenobarbitone, or Gardona at levels of 1.6, 16, 160, 1600, or 16,000 ppm. Animals were fed for 14 days. Random selections of mice from the groups were made for either morphological or biochemical studies.

a. Biochemical

Four mice in negative control, positive control and 16,000 ppm treatment groups, and 8 mice in each of the other groups were examined for protein, DNA, PNOD and G-6-P in liver homogenate.

b. Morphology

Livers of four animals/group were examined by light microscopy and E.M.

#2. Body and liver weights were studied in 6 males treated at 16,000 ppm Gardona and in 6 control animals.

4 males of each of these groups were also examined by light and electron microscopy.

#3. A feeding experiment with control diet, 160, 1600, 8,000 or 16,00 ppm of Gardona for 14 days was carried out.

A paired feeding experiment was also carried out on the 16,000 ppm dosage.

Food intake and body weight parameters were obtained on days 1, 2, 3, 4, 7, 11, 14.

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Methods:Light and E.M.

Animals were killed by i.p. pentobarbital. The livers were flushed with saline and perfused with 2.5% glutaraldehyde in Sorensens phosphate buffer for 5 minutes.

Post-fixation followed in either 10% formalin with routine processing with H&E staining (light microscopy) or in a fresh glutaraldehyde solution.

Liver Homogenates

Liver tissues were weighed and chilled in 0.25 M sucrose, 3 mM Tris HCl pH 7.4 at 0°C.

Both homogenate and microsomal suspensions were routinely made.

Assays:DNA:

Liver homogenate was precipitated with 0.5 N perchloric acid and sedimented in the cold, 0°C.

DNA was analyzed by the diphenylamine method of Burton, (1956).

Protein:

The method of Lowry et al. was used for liver & microsomal preparations.

G-6-P:

The methods of Harper (1963) and Fiske and Subbarow (1925) as modified for these preparations were used in the analysis for inorganic phosphorus.

PNOD (p-Nitroanisole-O-demethylase): was determined using the end point of enzymatic activity as the production of p-nitrophenol. Boiled and unboiled tissue preparations were analyzed.

Feed

Test materials were added to the feed as a solution in acetone. "Diets were made individually as required" stated to be e.g. - 5 days before commencement of feeding and analyzed for Gardona as appropriate.

A + 10% of nominal concentration was set.

Food intake was determined.

Statistics:

Liver and body weights were tested for covariance. Biochemistries, liver and body weight were examined by an analysis of variance. Positives were also examined with the t-test. Liver homogenate and microsomal preparation results were correlated by least squares analysis.

William's test was used to determine significance in the paired feeding portion of the study.

Results:

Experiment 1:

PNOD activity: (controls vs. 16,000 ppm and phenobarbital):

Gardona and phenobarbital activities were essentially equivalent and both were significantly increased at 16,000 and 500 ppm respectively when compared to controls ($p < 0.01$ or $p > 0.001$) for homogenate and microsomes. A S.D. for the 4 control animals was not presented.

The majority of the PNOD activity appeared to be present in the microsomes. Specific activity increased from approx. $2.19 \times$ - $2.62 \times$ over control with phenobarbital and Gardona respectively.

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G-6-Phosphatase activity:

There appeared to be a slight reduction in activity (not statistically significant) at 1600 ppm, but significant at 16,000 ppm.

S.D. values for controls were again missing and are requested for review.

The activity/gram of liver at 16,000 ppm was indicated as significant ($p < .05$) but can't be verified with the data presented.

G-6-P Activity per mg/DNA was not significantly different than controls except for phenobarbital; which showed a small increase ($p < .05$).

Experiment 2

PNOD activity at 1,600 ppm and above showed a significant increase in PNOD activity ($p < .001$) (compared to controls). Control values were presented without a S.D. or S.E. for comparison to treated values. A slight dose response compared to controls was exhibited as an increase in DNA/g liver (not significant) until the 1600 ppm level. At 1600 ppm and above a reduction in mg DNA/g liver occurred becoming statistically significant at 16,000 ppm ($p < .05$).

DNA Content of Liver Protein

Liver protein exhibited essentially the inverse of the DNA production/g of liver above. However the increase was not significant at any level. S.D. values for controls were omitted.

Mean body wts. are reduced at 8,000 ppm and above from day 2 to termination. A single day (4) showed a significant reduction in body wt. at 1,600 ppm when compared to controls. A real difference of greater than 10% wt. reduction in the pair fed 16,000 ppm group is seen. There appears to be a lowered wt. gain at 8000 ppm which increases by day 7. When comparing the daily food intake with controls, a slight increase in consumption occurs following day 5 and becomes more apparent after day 10 at 8,000 ppm.

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Conclusion

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A lowered feed utilization occurs at 8,000 ppm. It is not obvious to this reviewer why a pairedfeeding study group (16,000 ppm) would not be the same or larger than the corresponding control. A picture or drawing of the feeding apparatus used is requested since data suggests a residue was left. It is unknown whether the animals were able to reach all the minimized pair feed with the special hopper apparatus or not.

Morphology

In table 1 - the experimental design showed that 4 animals from each dosage group would be examined. However, appendix 4 indicates that in all groups excepting #5, 1,600 ppm at least 1 animal and as many as three produced poor sections for E.M.. Group #5 did not have sufficient animals for sampling of tissues for E.M. survey. Group 4 also did not have a sufficient number of treated animals for survey.

Comment

Since the thrust of this study appears to be in conjunction with the 2-year mouse feeding oncogenicity study to show a LEL and NOEL, there appear to be several data points lacking. The fact that 8,000 ppm was previously considered to be excessive, why then was that level missing and only the top value of 16,000 ppm used? The use of E.M. in surveying treated animals for a NOEL is hardly worthwhile when viewing only 2 animals; and especially so when such an increase in numbers is used at the highest dosage.

It is not evident just how the E.M. and light microscopy data was quantitated. 1) How many grids were observed, 2) What degree of proliferation was present, 3) How many different tissue blocks were cut from each animal, 4) How many representative areas of the liver/s were sampled?

In Summary

One can say that the E.M. shows changes at 16,000 ppm, and that data presented do not provide sufficient evidence that several of the animals treated at 1,600 ppm may not have had some degree of lesion. In addition a very major data point, that of 8,000 ppm, was not evaluated.

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At present there is insufficient evidence to support the premise that the liver changes observed are not precancerous changes or would reverse following removal of the test compound from the animals diet. While the original doses administered (8,000 and 16,000 ppm) are arguably excessive they did elicit morphological liver changes, which this data (14 days) appears to support. Whether these changes are clearly indicative of carcinogenic potential is debatable. They nonetheless are compound related.

Henry Spencer, Ph.D. *Handwritten: 1/25/83.*
Review Section #1
Toxicology Branch/HED (TS-769)

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