CASWELL FILE

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Pathology Working Group Slide Reevaluation of Livers of Rats in a 30-Month Oral Exposure to Chlordane - Accession No. 404337-01 (3 Volumes)

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FROM: Henry Spencer, Ph.D., Pharmacologist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: George T. LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Review Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

Theodore M. Farber, Ph.D., D.A.B.T., Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Background

Following the submission of a 30-month chronic feeding/oncogenicity study in Fischer 344 rats, the Agency reviews by the Office of Pesticides Programs and the Cancer Assessment Group of these data indicated that male rats at the highest dosage exhibited an increase in liver tumors.

The registrant, Velsicol Chemical Company, subsequently convened the Pathology Working Group (PWG) to reevaluate the slides of livers of the chlordane-treated rats reported in Accession No. 252267. This submission, Accession No. 404337-01, is that reevaluation.

Conclusions and Recommendations

1. The Toxicology Branch (TB) concludes that there was not a statistically significant increase in tumors in male rats after reevaluation by the PWG.

2. TB, in retrospect, concludes that excessive numbers of mammary tissues in all female test groups are missing in the histopathology report. This finding is generally sufficient to cause a downgrading of a study to Core Supplementary for oncogenicity. This action of downgrading the study is taken in spite of the fact that Dr. D.G. Goodman, D.V.M., of the PWG stated in conversation that "Almost all tumors of the mammary gland are first found grossly and that the additional numbers of adenomas, fibroadenomas, and adenocarcenomas that are found by tissue slide examination are minuscule." Dr. Goodman additionally notes that if the tissues were examined grossly, then the number of tumors reported would closely represent the number of animals grossly examined and found with a mammary tumor.

3. An MTD was not established in the study and this alone would support the downgrading of the study to Supplementary for the data requirement (83-2 oncogenicity).

4. The reevaluation by the PWG of the liver lesions, i.e., hypertrophy, indicates that the female and not the male rat exhibits the NOEL of 1 ppm and an LEL of 5.0 ppm under data requirement 83-1. The NOEL for males is 25 ppm (HDT).

5. Based on this review by TB, it is concluded that:

   a. A NOEL of 1 ppm for liver effects can be established in female rats based on an LEL for regional liver hypertrophy at 5 ppm.

   b. The oncogenicity previously noted in male rat livers is downgraded to a nonstatistical neoplastic increase at only the highest dosage of 25 ppm (Table 2).

c. The lack of an MTD in either sex at the 25 ppm (HDT) and the failure to report a large number of mammary tissues (slides) in females support the downgrading of the oncogenicity portion of the study to Core-Supplementary. This study does not fulfill registration requirements for oncogenicity testing.

6. The Agency questions how the Japanese report concluded that NOEL in the liver was based on effects in the male when the PWG clearly showed that the NOEL was based on liver effects in the female.

7. The Agency recommends that the registrant obtain and compare copies of the written Japanese criteria for liver pathology with the written criteria noted by Dr. R.M. Saurer and the PWG in their blind evaluation of the liver hypertrophy lesions.

Review

Velsicol Chemical Company convened a U.S. PWG to reevaluate the liver pathology noted in the initial study report by the Japanese contract laboratory, The Research Institute for Animal Science in Biochemistry and Toxicology (RIASBT), dated December 1, 1983, on the Thirty-Month Chronic Toxicity and Tumorigenicity Test in Rats with Chlordane Technical.

Charles River Fischer 344 rats from the Japanese supplier were fed diets of 0, 1, 5, 25 ppm of technical chlordane for 130 weeks.

Dose levels to be used in the chronic study were based on results of a 30-day feeding study utilizing 0, 50, 100, 200, 400, and 800 ppm. The histopathological lesion of "hepatocellular swelling" at 50 ppm was the end point on which the dosages were chosen.

Data presented in the original report show that mortality at 104 weeks was similar in treatment levels of both sexes. By the end of the study, males had succumbed in greater numbers than females. However, the controls also showed an increase in males dying compared to control females. Therefore, the increased death rate is difficult to ascribe to chlordane exposure.

Mean body weight gains or losses at the highest doses of 5 and 25 ppm were not grossly different from those of the control group animals; and only sporadically were statistically significant differences noted for weight loss at the highest

dosage level in females. This kind of weight loss change suggests only minimal toxicity to females at 25 ppm. Differences in water and food intake values in either sex were not biologically significant throughout the study.

Changes are noted in a few biochemical parameters in males, and include elevated serum inorganic phosphorus in the 5 and 25 ppm test groups at 52 weeks. However, only the 25 ppm group values remained elevated at the terminal sacrifice, although the elevation was not statistically significant. Females also exhibited elevated mean phosphorus values at 25 ppm at 52 and 130 weeks.

Other changes in mean biochemical parameters that are statistically significantly different from control values are so sporadic as to not be ascribable to treatment alone. Bilirubin value changes are not considered to be significant with the exception of males at 5 and 25 ppm at 130 weeks of treatment. Many leukemic animals are noted in pathological gross evaluations as being icteric. It would take only a small number of animals with icteric serum to influence the bilirubin values for individual test dosage groups. Therefore, this reviewer considers that bilirubin value increases not to be the result of the test compound. Liver enzymes often show individual values which appear increased. However, the test groups on the whole are so varied as to preclude showing mean group changes that are biologically or statistically significant.

Pathology:

Males

The original pathologist found a large number of male rats with leukemia. Coincidentially with the leukemia a number of animals were also diagnosed microscopically with a liver lesion(s) which included the terms: focal hyperplasia and/or hepatocellular swelling. The incidences of these lesions without leukemia are shown below for the male rats.

Focal Hyperplasia

0 ppm	1 ppm	5 ppm	25 ppm
3	4	3	1

Hepatocellular Swelling

0 ppm	1 ppm	5 ppm	25 ppm
1	0	0	6

These data do not represent an effect below 25 ppm in the liver of male rats.

Females

Focal Hyperplasia

0 ppm	1 ppm	5 ppm	25 ppm
4	8	1	0

Hepatocellular Swelling

0 ppm	1 ppm	5 ppm	25 ppm
1	1	1	26

These data indicate that females have this microscopic lesion only at 25 ppm.

This reviewer finds that incidental numbers of small tissues (i.e., parathyroid) usually included as a second or an adherent tissue were missed in sectioning.

The original study report lists all mammary glands as having been examined. However, when this reviewer examined the individual animal histopathology report, it was noted that a large percentage in each test group were not "in section." See the incidence below.

Mammary Glands Not in Section on Slide

0 ppm	1 ppm	5 ppm	25 ppm
25 (64)	25 (64)	25 (64)	21 (64)
39%			33%

( ) = Total animals in study group examined grossly.

There also appears to be an increase in the numbers of subcutaneous neoplasms; however, when the individual sites and tumor types are separated, statistical significance is not achieved or approached.

This study is core classified for oncogencity as SUPPLEMENTARY due to:

1. The lack of an MTD at the highest dosage level of 25 ppm. This is based on no significant consistent losses in body weight, only microscopic lesions of probably a nonlife-threatening nature and the absence of other major toxicity endpoints; and

2. Additionally, although to a lesser extent but nonetheless valid, the fact that so many specific tissues (mammary) are missing (33 to 39%) in all test groups of the same sex does not allow for a complete examination of the oncogenic potential for the female rat in the study, even though most neoplasms of the mammary tissues may be first found by gross examination.

The rereview of the liver tissues of the rat study by Clements-Pathco is noted in the following pages.

Review and Conclusions of the PWG

The consulting firm of IFC-Clement, Fairfax, Va., organized a Pathology Peer Review of Chlordane in F344 Rats which was composed of the following pathologists.

Reviewing (Quality Assessment) Pathologist

Miriam R. Anver, D.V.M., Ph.D.

Pathology Working Group

Dawn G. Goodman, V.M.D., Chairperson
Alexander W. Macklin, D.V.M., Ph.D.
Robert R. Maranpot, D.V.M., M.P.H.
James A. Popp, D.V.M., Ph.D.
Robert A. Squire, D.V.M., Ph.D.
Jerrold M. Ward, D.V.M., Ph.D.

Pathologist for "Blind" Review of Livers for Hepatocellular Hypertrophy

Robert M. Sauer, V.M.D.

This review group (PWG) was convened because of the apparent increase of liver neoplasms (hepatocellular adenomas) occurring in older male rats exposed to chlordane (see Table 1). In addition to neoplasms, there was a significant increase in hepatocellular swelling or focal enlargement in the males

at all test dose levels. A no-observed-effect level (NOEL) was not established for this effect, hepatocellular swelling or focal enlargement, in the original review of the study.

The original study submitted by RIABST to Velsicol Chemical Company reported several toxic endpoints as histopathologic occurrences. These data have since been reviewed by the EPA reviewing contractor (Dynamac) and the Cancer Assessment Group (CAG) of EPA. In both instances, assuming the histopathology report to be correct and without benefit of confirmation of the slide lesions, the male rat was found to exhibit: 1) increases in hepatocellular adenomas, and 2) hepatocellular swelling/focal hepatocellular enlargement and necrosis.

The male rat data did not allow the determination of a NOEL at the low dose tested of 1 ppm (Table 1).

Unlike the male data, in the original report only the highest dosage level of 25 ppm in females was considered to be an LEL with a NOEL established at 5 ppm for hepatocellular changes.

The registrant commissioned a rereview of the liver slides which was carried out in several steps. The first step was to have M.R. Anver D.V.M., Ph.D., evaluate all the liver slides and note any discrepancies found in the RIASBT pathologist's report.

The PWG further resolved all discrepancies noted by Dr. Anver by evaluating the slides on a coded "blind" basis without knowledge of dose group, age, sex, or discrepancies. Evaluation was made by consensus of the six members. The PWG noted that they differed from the original RIASBT report with regard to the incidence of proliferative lesions of the liver. See Tables 1 and 2.

When the reevaluated neoplastic lesions in the male livers are summed, it is noted that there is a lack of statistical significance when comparing the incidence in the highest dosage group to that of the controls (Table 2). However, there is an apparent increase of adenomas at the 25 ppm dosage level over control values.

In order to more properly evaluate this increase in adenoma incidence, this reviewer examined the reported historical control data for the laboratory (RIABST). In one set of studies totaling 320 male rats, an incidence of 8 animals exhibited liver adenomas (2.5%). A second set of data (1 study) showed males with a 9.6 percent incidence of

Table 1*

Liver Tumors and Nonneoplastic Lesions In
F344 Rats Fed Chlordane (RIASBT 1983)

(Number of Animals Examined Per Dose Group is 64[a])

	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	25	0	1	5	25
Hepatocellular adenoma	1	1	3	9	0	2	0	0
Hepatocellular swelling/focal hepatocellular enlargement	7	16	16	43	8	5	5	37
Necrosis (includes NOS[b], focal, and hepatocellular)	13	22	21	39	8	4	4	10
Hepatocellular fatty degeneration	26	13	20	23	20	16	19	20

[a]Excludes 16 interim kill animals per group.
[b]Not otherwise specified.
*Extracted from PWG report.

Table 2**

Proliferative Hepatocellular Lesions

(Number of Animals Examined is 64)

	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	25	0	1	5	25
Hepatocellular adenoma	2 (3%)	4 (6%)	2 (3%)	7* (11%)	0	1	0	0
Foci of cellular alteration[a]	8	16	14	8	28	35	32	32

*Not significant, Fisher's exact test, one-tailed (p = 0.08)
[a]Nonleukemic livers.
**Extracted from PWG report.

hepatocellular adenomas in a study of 42 males. It is assumed that both sets of data covered a lifetime of at least 105 weeks. An open literature report by H.A. Solleveld indicates that neoplasms increase markedly after 105 weeks in the F344 rat.

Lifespan data on male F344 rats shown by Solleveld indicate that at least 8.6 percent of 529 rats were diagnosed with neoplastic nodules. He reported that males within the 124 to 136 weeks age group could show as much as a 14 percent incidence of neoplastic nodules. Therefore, the 11 percent incidence of adenomas at the highest dose (25 ppm), although slightly increased above controls, is not considered by this reviewer to be the likely result of chemical treatment, but rather the result of excessively aged animals. These data are even more in doubt of a positive chemical effect because the controls appeared to have had a below normal incidence of adenomas for life study animals (3%versus 9.6%).

An additional consideration by the PWG pertained to hepatocellular hypertrophy. Robert M. Sauer, V.M.D., was contracted to read all liver slides of animals without leukemia.

The following extracted information is included from the PWG report** concerning nonproliferative lesions of the liver.

It is important to note that the PWG report considered the RIASBT pathologist as having inconsistently diagnosed hypertrophy and degenerative lesions. It was for that reason that the criteria were included below for consistency in evaluating these lesions.

**Nonproliferative Lesions. Because the RIASBT study was a 30-month feeding study and survival was not compromised by the administration of chlordane, many of the aging F344 rats, particularly the males, developed spontaneous leukemia. The pathology related to this tumor has been well described (Stromberg and Vogtsberger 1983) and the tumor cell characterized as a large granular lymphocyte (Ward and Reynolds 1983). Elevated incidences of mononuclear cell leukemia have been reported in F344 control rats when allowed to live beyond 104 weeks compared with control F344 rats terminated at 104 weeks. [Solleveld et al. (1984).]

Liver lesions secondary to leukemia have been reported to occur frequently; such lesions include necrosis, cytomegaly (swelling/hypertrophy), and fatty change (degeneration) (Stromberg and Vogtsbergr 1983). Diagnosing these secondary

lesions can add a great deal of uncertainty to lesion incidence tables, since such lesions cannot be attributed to toxic effects induced by a specific compound.

The original (RIASBT) pathologist (OP) for this study inconsistently diagnosed these secondary lesions. For some animals, in all dose groups, he diagnosed necrosis, swelling, and/or fatty degeneration in leukemia livers, while in other leukemia livers, he diagnosed leukemia and no additional degenerative lesions, even though such were present.

For every OP diagnosis of intercurrent degenerative lesions in liver with leukemia, as well as in a smaller number of rats with histiocytic sarcoma of the liver, the consensus of the PWG was that these nonneoplastic liver lesions were secondary to the leukemia infiltrates and that they should not be diagnosed as separate entities.

The following paragraphs represent the diagnostic criteria* used by the PWG and have been extracted from their report.

*Hepatocellular Hypertrophy. This nonproliferative lesion was characterized by the presence of large hepatocytes with eosinophilic cytoplasm. The PWG preferred the term "hypertrophy" over "swelling" or "cytomegaly." Hepatocellular hypertrophy in a liver with leukemic infiltrates was considered to be a secondary change which the PWG felt should not be diagnosed as a separate entity.

*Regional hepatocellular hypertrophy was associated with dietary administration of chlordane. Based on PWG recommendations, a "blind" review of livers was performed to determine the incidence of this lesion. The population of animals examined for this lesion was composed of rats which did not have diagnoses of leukemia by either the original or PWG reviewing pathologist or histiocytic sarcoma by the PWG reviewing pathologist in the liver.

*Regional hepatocellular hypertrophy consisted of a group of three or more contiguous liver lobules in which there was centrilobular hepatocellular hypertrophy. The hypertrophy frequently extended peripherally to the portal triads. The resultant confluent group of hypertrophied lobules formed a rather discrete area that mildly compressed adjacent parenchyma at some point on the circumference. The integrity of each lobule was maintained within the affected area. Hypertrophied hepatocytes were moderately to greatly enlarged and contained cytoplasm, which often had increased eosinophilia. Nuclei were usually mildly to moderately enlarged but otherwise were normal in appearance.

These regional areas of lobular hypertrophy were usually solitary but, if multiple, were in the order of two or three discrete lesions scattered throughout one or more liver sections. There did not appear to be a predilection for any particular lobe of the liver.

The incidence of this lesion is in Table 3.

"Thus, regional hepatocellular hypertrophy, a nonlife-threatening lesion, was associated with administration of chlordane for 30 months in female rats fed 5 and 25 ppm. This lesion also was present spontaneously (in control females). The PWG noted that this regional pattern of hypertrophy was not the type generally associated with other compounds affecting the liver and therefore could not be certain as to whether this mild lesion represented a hepatotoxic response to chlordane."

Table 3

Regional Hepatocellular Hypertrophy

	Dose Group (ppm)			
	0	1	5	25
Females[a]:				
No. livers examined	37	43	40	44
No. with lesion	2	4	9	15
Fisher's exact test (1-tailed)	--	N.S.	p = .032	p = .0013
Males[b]:				
No. livers examined	20	23	20	16
No. with lesion	0	1	0	0

[a]Cochran-Armitage trend test (females): p = .0003.
[b]Statistical analyses not performed on incidences in males.
N.S.= Not significant.

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