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

004521

JUN 28 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Naled (DIBROM) - Appraisal of Company Response to EPA Reviews of Rat (S-1802) and Mouse (S-1664) Chronic Studies, Originally Submitted Under Acc. Nos. 254217 through 254224 and 254225 through 254228, respectively.
EPA Registration No. 239-1633 Caswell # 586

TO: William Miller/G. Otakie, PM-16
Registration Division (TS-767)

FROM: Irving Mauer, Ph.D.
Section VI
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THRU: Jane E. Harris, Ph.D.
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Following is a point-for-point appraisal of Chevron's response, under cover dated April 2, 1985, to the Agency's assessments (SUPPLEMENTARY) of the Lifetime Oral Carcinogenicity Study in Mice, S-1664 conducted at IRDC (#415-038), and the Chronic Oral Toxicity/Oncogenicity Study in Rats, S-1802, conducted at Bio-Research Labs (#9394).

Toxicology Branch Conclusions

Mouse study (S-1664): Upgraded to CORE MINIMUM

Rat study (S-1802): Upgraded to CORE MINIMUM for both chronic toxicity and oncogenicity.

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MOUSE STUDY

ISSUE 1: Was the HDT a MTD?

EPA Review: No rationale was given for the dosage choice (including gavage administration).

Chevron Rebuttal: The registrant submits with this response a range-finding study in mice conducted at IRDC (S-1663, dated August 21, 1980) inadvertently not transmitted with the chronic study, which recommended a high dose for the latter between 50 and 100 mg/kg/day based upon severe cholinergic effects and death at 300 mg/kg/day, tremors and reduced motor activity in one 150 mg/kg/day female, and statistically significant, dose-related decreases in food consumed by 20 and 50 mg/kg/day animals.

Agency Response: We find the data presented in this range-finding study justified the selection of 75 mg/kg/day as the HDT for the chronic study (see DATA REVIEW for range-finding assay attached to this memo).

We also note that the gavage route was chosen for these studies because of significant degradation of Dibrom incorporated into feed (as indicated in Appendix 1 of the company's response), which point is acceptable.

ISSUE 2: Did the "steep dose-response curve for mortality" compromise the study?

EPA Review: The maximum tolerated dose may not have been approached, and there appeared to be insufficient rationale for decreasing the top dose from 75 to 50 mg/kg/day at week 27.

Chevron Rebuttal: The number of high-dose (75 mg/kg/day) animals that died (11 by week 13, and 3 more by week 26) constituted an "...alarming number of deaths so early into a chronic study..." and even in the absence of serious clinical distress, were considered compound-related. Further, decreases in body weights (especially in males) by at least 6 percent at 27 of 51 weighing intervals, 9 percent at week 16 and 8 percent at week 40 also constitute real compound effects.

Agency Response: In the light the data presented in the range-finding study, we have re-evaluated the chronic, noting:

(i) Dose-selection was appropriate

(ii) Increased mortality at week-26 for both HDT males and females, and tremors in 3 of the 8 females dying at 75 mg/kg/day, justified reduction in dosage to 50 mg/kg/day from week-27 on.

(iii) At 89 weeks, mortality was still higher in treated males, and indicative of severe effects at 50 mg/kg/day.

(iv) Fifty mg/kg/day represents a high oral dosage level in mice, considering published acute studies which give LD_{50} values ranging from 160 mg/kg (Berteau and Been, BULL. ENVIRON. CONTAM. TOXICOL. 19:113, 1978) to 330 mg/kg (NIOSH, Registry of Toxic Substances, 1979).

ISSUES 3 and 4: Did termination of the study at 89 weeks when unexplained mortality in control females approached 50 percent compromise the detection of an oncogenic effect (as stated in the EPA review)?

Chevron Rebuttal: The registrant states that in high dose females with higher liver/body weight ratios, "no concomitant histopathologic changes at 21 months indicated that these liver weight changes were probably random in nature and not related to treatment." Further, the mortality in control females at 21 months was not considered "excessive" but "...indicative of the animals' normal lifespan."

EPA Response: The company's rebuttal is accepted. Further, the Agency's current Pesticide Assessment Guidelines (Subdivision F, Section 83-2) permit termination of an oncogenicity study in mice during the interval 18 to 24 months

ISSUE 5: Were the two batches of test material equivalent to "technical grade Dibrom" (especially the purity of that used in the second year of the study)?

Chevron Rebuttal: The registrant has provided in his response an attachment (Appendix 2) analytical data indicating both "batches" were from the same formulation, SX-1203, but differently labeled. This issue is resolved.

Agency Conclusion: The chronic mouse study (S-1664) is reclassified as CORE-MINIMUM.

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RAT STUDY

[Note: The registrant has submitted with his response: i) a 28-day range-finding study in rats (also conducted at Bio-Research) upon which dosage selection for the Rat Chronic Study was based (see Tox Branch Data Review attached to this memo); ii) a dietary stability study of DIBROM incorporated into standard rodent feed (as Appendix II to the company reponse), indicating that the technical degrades rapidly at room temperature (half-life of 1.5 days at 21 °C) irrespective of concentration, and thus justified the use of gavage; and iii) four acute oral studies of DIBROM (as Appendix III of the company response). The Agency has considered these submissions in the following appraisal of Chevron's rebuttal to our review and classification of the rat chronic study.]

MAJOR ISSUE: Was an MTD approached?

EPA Review: The high dose (10 mg/kg/day) was only 2 to 4 percent of the LD_{50} reported in the available literature.

Company Rebuttal: The acute studies appended (representing a total of five tests) indicate that DIBROM was more toxic when administered as an aqueous suspension in 0.5 percent CMC (in two tests, LD_{50} males, females = 191 and 85 mg/kg, 92 and 81 mg/kg) than corn oil preparations (LD_{50} in males ranged from 325 to 386 mg/kg, in females 207 to 236 mg/kg). The literature cites an LD_{50} of approximately 430 mg/kg in the rat using corn oil as the vehicle. Thus the HDT in the rat chronic study (which also used CMC as the vehicle) was 5 to 12 percent of the LD_{50} for males and approximately 12 percent for females. Further, the high dose for the chronic study (10 mg/kg/day) was chosen on the basis of significant depression of cholinesterase values at this dose in a pilot study, approximately 20 to 25 percent for erythrocytes and 50 percent for brain. Similar reductions in rbc's were found in high-dose animals during the course of the chronic study, as well as in brain at the termination of the study.

EPA Response: The Agency has reviewed the pilot rat study (see Data Review attached to this memo), and finds acceptable justification for the dose selection in the chronic. We also note the increased acute toxicity of carboxymethyl cellulose over corn oil preparations, as indicated in the studies submitted as Appendix III (which were previously reviewed-Memo, Mauer to Miller, December 24, 1984). Thus, the issue on the MTD level is resolved.

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Minor Issues: The following minor deficiencies culled by the registrant from the Agency's review of the rat chronic study are also resolved by acceptable explanations provided in the company response (Chevron):

1. "Individual clinical observation data were not present." (The raw data are available for audit or review as photocopies.)

2. "There were no interim (52 week) sacrifice animals." (None are required.)

3. "The thoroughness of the gross pathological evaluation could not be determined." (Complete individual gross findings are available as Appendices 16 to 21 of the report.)

4. "Brain cholinesterase activity was determined from only one hemisphere, not the whole brain." (Since both post mortem enzymatic analysis and microscopic evaluation cannot be performed on the same tissue sample, the left hemispheres were homogenized for determination of cholinesterase activity, and three separate histological sections were taken from right hemispheres for histopathological examination.)

5. "It was not specified whether only one or both adrenals of each animal were examined." (Both adrenals were routinely evaluated, unless one was lost or damaged during handling.)

6. "There was no apparent explanation present for the increased mortality of female control animals at the end of the study." (Since there was no apparent reason for this increased mortality, it may be random or spurious. The evaluation of pathological data for control females which died prior to terminal sacrifice gave evidence of pituitary adenoma, commonly seen in aged S-D rats.)

Agency Conclusion: The combined rat study (S-1802) is reclassified as CORE MINIMUM for both chronic toxicity and oncogenicity.

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TOXICOLOGY BRANCH: DATA REVIEW

Caswell 586
EPA Chem: 034401

Chemical: Naled (DIBROM)

Study Type: Subchronic (range-finding) Oral --- Mouse

Citation: Technical Dibrom: 4-Week Dose Range-Finding Study in Mice (Extended to 6 Weeks)

Accession No./MRID No.: NA

Sponsor/Testing Lab: Chevron/IRDC

Study No./ Date: S-1663/August 21, 1980

Test Material: Technical Dibrom, SX-1203 (92.7% ai) a solid, white, waxy substance suspended in 0.5% CMC for oral administration.

Procedures: CD-1 mice (10 per sex per group) were gavaged daily for 4 weeks with 0(CMC), 1, 5, 20 and 50 mg/kg test material. The dosage in the two lower test groups was increased to 150 and 300 mg/kd/day for a further 2 weeks, while that in the two higher groups maintained. Animals were observed 2 to 3 times daily, weighed weekly, and food consumption measured weekly. All animals were examined grossly for pathological lesions, tissues fixed in formalin, but no histopathology performed.

Results: During the first 4 weeks of treatment, no adverse clinical effects were observed in any group. One 1 mg/kg/day male died in week 3, reportedly from "mechanical injury." During the subsequent 2-week treatment, dose-related effects were noted, especially severe in the test group in which dosage had been increased to 300 mg/kg/day. These included tremors (4 males, 4 females), reduced motor activity (5,3), yellow material around mouth (4,2), lacrimation (4,2), white material around eyes (2,1), yellow-brown anogenital staining (2 males), salivation (2,1), and death within 5 days of dose increase (6,8). Only one female in the 150 mg/kg/day group exhibited tremors and reduced motor activity. After 6 weeks treatment, only slight (insignificant) decreases in mean body weights compared to controls were recorded in the 50 (-5.9 gm), 150 (-2.9 gm) and 300 (-2.9) mg/kg groups, but food consumption was significantly less than controls for the 20 ($p < 0.05$) and 50 ($p < 0.01$) mg/kg groups (-3.7 gm/mouse/day for each).

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No gross pathological lesions indicative of a compound-related effect were noted in the 300 mg/kg group animals which died or were sacrificed, but a statistically significant, though slight, increase in kidney weight compared to controls was noted in the two remaining females of this group sacrificed at 6 weeks (absolute, from 0.42 to 0.54 gm; relative, from 1.70 to 2.08% of body weight.) No other alterations were reported in any test group.

Conclusions: On the basis of this range-finding study, the authors recommended that " ...the high-dose level for the succeding oncogenicity study in mice between 50 and 100 mg/kg/day to avoid excessive toxicity and mortality." [Note: 75 mg/kg/day was the HDT chosen for the oncogenicity study, Acc. Nos. 254225-254228--see evaluation of this study, attached to memo: Mauer to Miller, dated December 20, 1984.]

TB Evaluation: The 300 mg/kg/day level was certainly an effect level causing severe toxicity and death (60-80% mortality) within two weeks, but the duration (2-week) of dosage at 150 mg/kg would appear to be too short to categorize this level as excessively toxic, considering only one female in this test group manifested mild cholinergic effects.

SUPPLEMENTARY DATA

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TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Naled (DIBROM) Caswell: 386
EPA Chem.#: 034401

Study Type: Subchronic Oral (range-finding)--Rat

Citation: DIBROM. Four-Week Subchronic Oral Toxicity Study in Rats.

n,cession No./MRID No.: NA

Sponsor/Testing Lab: Chevron/Bio-Research Lab

Study No./Date: BRL #9393 (Chevron #S-1801)/October 1, 1981

Test Material: Technical DIBROM (SX-1278, 92% ai), a yellow viscous liquid, suspended in 0.5 percent aqueous carboxy-methyl cellulose (CMC).

Procedures: Test material was administered by gavage to 46-day old CD-Cr:(SD)BR albino rats (10/sex/group) at levels of 0 (CMC vehicle), 0.25, 1.0, 10, and 100 mg/kg/day (approximately 0.06%, 0.25%, 2.5%. and 25% of the LD_{50} for test groups, respectively) for 28 days. Animals were observed daily, weighed weekly, and food consumption recorded (and efficiency of utilization calculated) weekly. Circulating (plasma and erythrocyte) cholinesterase activities were determined from orbital sinus blood during the four weeks of treatment, and brain cholinesterase on survivors at sacrifice. All animals which died during the treatment period as well as all survivors were subjected to full gross pathology, and organ weights (absolute and relative) recorded for brain, heart, kidneys, liver, ovaries, and testes. Although no histopathological examinations were performed, the full array of tissues plus any abnormal tissues were fixed and retained.

Results: All or a majority of animals treated at or surviving 100 mg/kg/day manifested some degree of muscular tremor, lacrimation, and/or urogenital discharge on a majority of occasions within an hour or two of dosing (with recovery within 2 to 3 hours), while a (lesser) number showed evidence of general weakness, poor condition, pallor, lethargy, hypothermia, or respiratory distress after dosing. Intermittent ocular or nasal discharge as well as urogenital staining and loose moist feces were noted in several HDT animals on various occasions, progressively more frequent in the third and fourth weeks of treatment. A total of seven high-dose males and five females died during the study, macroscopic pathology revealing a variety of alterations, including pulmonary swelling and red or dark pinpoint foci, white tracheal froth, thickening of the jejunum,

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glandular stomach foci, and enlarged cervical lymph nodes (Appendices 1 through 4 and 19, 20 of report).

Among animals treated at 10 mg DIBROM/kg/day, one male showed muscular weakness after the sixth dose, and one other male and four females were slightly lethargic on the same occasion. One female of this group died after the sixth dose as a result of accidental perforation of the esophagus. No overt clinical reactions or deaths were noted among animals treated at 0.25 or 1.0 mg/kg/day.

Except for reduced body weight gain, food intake and utilization in HDT males (but not females) during the first week of treatment (with recovery by the fourth week), no ponderal or feeding differences from controls were found in any other test group (Figure I and II, Tables 2 through 9, Appendices 5 through 12).

After 4 wk treatment, comparably significant depressions were recorded in rbc cholinesterase activities of high-dose and 10 mg/kg/day group males and females (24% and 30% of control values). Reduction in plasma values, however, was dose-related at these levels (40 to 50% for males and females on 10 mg/kg/day, 50 to 75% at the HDT); as well, there were with slight reductions (15%) in 1 mg/kg/day females. Brain cholinesterase was depressed by 75% at the HDT and 50 percent at 10 mg/kg/day (Tables 10, 11, and Appendices 14 through 17).

No evidence of gross pathology was found in surviving animals (three males and five females at the HDT, all but one 10 mg/kg female at other levels) killed after 4 wk treatment (Appendices 19, 20). Except for both absolute and relative liver weight increases in surviving HDT females, but only group mean relative increases in surviving males at that dose, no other organ weight differences from controls were considered compound-related (Tables 12 through 15, Appendices 21 through 24).

Conclusions: Based upon the results of this study, the authors recommended that "...the high-dose level for the ...2-year study... should not exceed 10 mg/kg/day."

TB Evaluation: We concur that 100 mg/kg/day represents a severely toxic (and lethal) dose, and 10 mg/kg/day a minimally effective level based on a few mild cholinergic signs concomittant with (dose-related) suppression of plasma, erythrocyte and brain cholinesterase activities. SUPPLEMENTARY DATA.

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