10-25-85

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

004735

OCT 25 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Responses to the registrant's comments on issues raised by the Agency relative to the rabbit teratology and rat chronic/oncogenic studies with Technical Thiobencarb (Bolero)
EPA No. 239-2431
Accessions No. 258188 and 258189 Caswell No. 207 DA

TO: Richard Mountford, PM #23
Herbicide Fungicide Branch
Registration Division (TS-767C)

FROM: Quang Q. Bui, Ph.D. Quang Q. Bui 10/21/85
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Laurence D. Chitlikl, D.A.B.T. W. Tetters for L. Chitlikl 10-21-85
Section Head, Section V
Toxicology Branch/HED (TS-769C)

and

Theodore M. Farber, Ph.D. 10.24.85
Chief, Toxicology Branch
Hazard Evaluation Divison (TS-769C)

Registrant:
Chevron Chemical Co.
940 Hensley St.,
Richmond, CA. 94804

Action Requested:

Respond to the registrant's comments on specific issues raised by the Agency relative to the rabbit teratology study (Bio/dynamics #S-2241, 12/20/83) and rat chronic/oncogenic study (Life Science #83-KCI045/248, 7/84) with Technical Thiobencarb (Bolero).

RECOMMENDATION

1. Rabbit Teratology Study (Bio/dynamics Inc., # S-2241, 12/20/83)

It is rececommended that this study remain classified as Core Supplementary Data. A new study will be required to fully evaluate the potential developmental toxic effect of Bolero. It is suggested that a new rabbit teratology be submitted in which dosing of the does occurs only from gestational days 6-18, with their

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sacrifice on day 29 (see "Discussion" section on page 6 of this memo).

2. Rat chronic/oncogenic study (Life Science Research #83/KCI045/248, 9/7/84)

The rat chronic/oncogenic study is upgraded to Core Minimum Data with the following findings:

Systemic NOEL = 20 ppm (lowest dose tested)
Systemic LEL = 100 ppm (decreased food consumption, decreased body weight gain, increased blood urea nitrogen)
Carcinogenic potential negative up to and including the highest dosage level tested (500 ppm).

BACKGROUND INFORMATION

The rabbit teratology study (Bio/dynamics Inc., #S-2241) and the rat chronic/oncogenic study (Life Science #83-KCI045/248) were previously reviewed by the Agency and were classified as Core Supplementary Data (Q. Bui memos of 1/23/85 and 2/15/85). In this action, additional information requested by the Agency and clarification of several issues raised in the Agency's reviews were submitted by the registrant. Responses to the registrant's comments for each study are discussed separately in this memo.

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DISCUSSION

RABBIT TERATOLOGY STUDY (Bio/dynamics # S-2241, 12/20/83)

Three major issues were raised by the Agency's reviewer in his memo of 1/23/85. These are:

1. Premature delivery
2. Cannibalism
3. Conduct of the study

1. Premature Delivery

In this study, premature delivery was found in all groups (4/4), including the control. The premature delivery indices (defined as # dams premature delivery / # of pregnant females - # pregnant females that died) found in the 0, 2, 20, and 100 mg/kg group were, respectively, 5.9% (1/17 females), 20% (3/15 females), 14.3% (2/14 females), and 40% (6/15 females). In the final report, the investigators indicated a historical premature delivery incidence of 8.9%. However, data to substantiate their findings were not submitted with the final report, hence, the Agency's request for its submission.

In this action, historical control data from 10 main studies (11 control groups) and 12 pilot studies covering an interval of approximately 4 years were submitted. Calculation of the premature delivery index from the submitted historical control data resulted in the following:

		Premature Del. Index
Historical control, main studies (11 groups)		7/180 = 3.9%
Historical control, pilot studies (12 groups)		7/65 = 10.8%
Historical control, all studies combined (23 groups)		14/245 = 5.7%
Study S-2241	Concurrent control	1/17 = 5.9%
	2 mg/kg	3/15 = 20.0% *
	20 mg/kg	2/14 = 14.3%
	100 mg/kg	6/15 = 40.0% *

(*) Significantly different from historical control values (all studies combined), $P < 0.05$, Fischer's Exact Test, calculated by this reviewer.

The premature index calculated for the historical main control studies was 3.9% and that of all historical control data combined (main and pilot studies) was found to be 5.7%. Both of these values do not substantiate an incidence of 8.9%, the value given in the final report. The registrant is requested to provide explanation for this discrepancy. Regardless of which historical premature delivery index is used (3.9% or 5.7%), the values obtained in this study for the Bolero-treated groups (20.0%, 14.3%, and 40.0% for the 2, 20, and 100 mg/kg groups, respectively) are still higher than the historical control data with statistically significant differences found for the 2 and 100 mg/kg dosage levels. The concurrent control incidence of 5.9% is comparable to that of the historical control data. These findings collectively suggest that the high incidence of premature delivery observed in all treated groups apparently was compound-related.

The registrant stated that "the increased premature delivey index seen at the high-dose level in the Bolero teratology study may be suggestive of a treatment-related response" and agreed with this reviewer that "premature delivery may be due to many factors of which compound-induced effects and excessive maternal stress (gavaging, handling of animals during late pregnancy) are contributory". However, the investigators emphasized that in this study, all animals were dosed from days 7-29 following the 1978 Proposed Guidelines and "with this treatment regime, there is reasonable stress to the animal and the opportunity of compound-related toxicity to demonstrate a possible abortifacient effect is enhanced".

This reviewer disagrees with the investigators for the following reasons:

a) From the submitted historical control data, premature delivery apparently was not related to the dosing schedule as evidenced in the following table:

	Premature Delivery Index
-Dosing from days 7-19 (4 main groups and 5 pilot groups)	6/94 = 6.38%
-Dosing from days 7-29 (7 main groups and 7 pilot groups)	8/151 = 5.30%

Therefore, in consideration of these additional data, the higher premature delivery index observed in all Bolero-treated groups is even more suggestive of a compound-related effect. It should be noted that although both dosing schedules are acceptable to the Agency, dosing only during the period of organogenesis is preferred.

Issue No. 2: Cannibalism

In this study, cannibalism occurred only in the Bolero-treated groups. The cannibalism rates (ratios of dams with cannibalization/dams with premature delivery) for the 2, 20, and 100 mg/kg groups were, respectively, 67, 100, and 83%. A historical control incidence of 57% was given by the investigators. The total numbers of partially cannibalized pups found after premature delivery in the control, 2, 20, and 100 mg/kg groups were 0, 7, 3, and 26, respectively. However, it should be noted that these numbers referred only to a minimum number of pups that were cannibalized since only partially cannibalized pups were available for examination. The total number of pups likely cannibalized (sum of partially cannibalized and unaccounted implantations) may be 0, 13, 8, and 29, respectively.

The registrant indicated that "cannibalism in rabbits is frequently asociated with poor nest building, with young being outside the nest, or with disturbance of the doe" and concluded that "there is no evidence that the fetuses which were cannibalized were malformed, nor is there support for this view in the literature".

This reviewer agrees that the lack of nest building material, young being outside the nest, and disturbance of the doe may well be causative factors for cannibalism. However, this reviewer also recognizes that in a teratology study, cannibalism can occur only if there has been premature delivery; that is unless premature delivery occurs there is no possibility of having cannibalism. Therefore, in a teratology study, care should be exercised so that the pregnant animals do not suffer excessive handling or undue excitement or trauma. And, it is all investigators' responsibility to interupt pregnancy one full day prior to expected delivery to insure that all fetuses are collected for examination. In this study,

the main question is not whether cannibalism is associated with dead, weak, or malformed fetuses or what causes cannibalism but should concentrate, rather on, what impact the presence of cannibalism has on interpretation of the data.

It is clear from the data submitted that premature deliveries followed by cannibalism in the treated groups undoubtedly restrict the total number of litters and pups available for examination, limit the scientific significance of the data evaluated, and lead to uncertainties relative to the possible developmental toxic effects of Bolero on the "cannibalized" pups. Due to excessive cannibalization in the 100 mg/kg group, the total number of litters collected for this group was reduced to 9, which is lower than the requirement of 12 litters per dose level.

Issue # 3: Study Conduct

In this study, there are many factors which collectively led to this reviewer's statement of "poor conduct and/or supervision of the study":

a. Premature delivery in all groups including the control
b. Presence of cannibalism in all treated groups
c. Unrecorded viability status of 10 pups in the 20 mg/kg group
d. 5 fetuses from one control litter were disarticulated during the staining procedure and were excluded from the evaluation

This reviewer never questioned the handling of animals and dosing procedures of the investigators, but stated that one of the factors that may lead to premature delivery is excessive maternal stress due to gavaging, which necessitates handling of the animals during late pregnancy (as performed in this study). However, the absence of an adequate explanation in the final report relative to premature delivery and cannibalism, coupled with the lack of information concerning room checks, laboratory supervision, and quality assurance, justified the questioning of adequate conduct and/or supervision of the study.

In this action, information relative to room checks, laboratory supervision, and quality assurance were submitted. With respect to "unrecorded viability status" and "disarticulated fetuses", the investigators stated that "the technician should have recorded the status of the pups" and "it is good to record such information but these data have little use in extrapolation to the in utero data", as well as, "it is unfortunate that this disarticulation of specimens occurred". From a scientific point of view, this reviewer concurs that the "unrecorded viability status of 10 pups" and the loss of skeletal data from 5 control fetuses may not have a significant impact on the interpretation of the data, but from a technical aspect, these issues should be mentioned. In fact, it is customary in a teratology not to use data from premature deliveries, abortions, and dead animals in the calculation of both maternal and in utero fetal data and this approach was applied by this reviewer in the evaluation of this study. However, data from animals which prematurely deliver should still be evaluated, but separately.

DISCUSSION

In addition to the comments relative to the issues raised by the Agency, the registrant also submitted historical control data for malformations, individual skeletal malformations and variations for fetuses obtained at cesarean section, as well as individual data for fetuses recovered from premature delivery. The mean fetal and litter incidences for each finding were also provided.

From the newly submitted data, the premature delivery incidences found in all Bolero-treated groups are higher than those of the historical control data (20.0%, 14.3%, and 40.0% for the 2, 20, and 100 mg/kg groups, respectively, as compared to a historical control incidence of 5.7%) and are suggestive of a compound-related effect. As indicated by the registrant, "the increase in premature delivery in the 100 mg/kg group may have been related to the administration of Bolero"; the premature incidences in the 2 and 20 mg/kg groups also may well be compound-related since these incidences are much higher than both the concurrent and historical control data. If such is the case, maternal toxicity was demonstrated at all dose levels, including the lowest dose used (NOEL < 2 mg/kg/day). It is agreed that, in addition to compound-related effects, many other external factors such as excessive maternal stress may have contributed to the premature delivery observed in the treated animals. However, all investigators should strive to reduce unnecessary maternal stress (for example, by limiting the dosing period just to the duration of organogenesis) and to avoid any external factors which may enhance the compound effects on the dams.

Furthermore, due to premature deliveries and ensuing cannibalism in the treated groups, the number of litters available for evaluation in the 100 mg/kg group was reduced to 9, resulting in unneccessary loss of fetal data and decreasing the number of litters available for evaluation below that required by FIFRA Guidelines.

It is recommended that this study remain classified as Core Supplementary Data. A new study will be required to fully evaluate the potential developmental toxic effect of Bolero. It is suggested that a new rabbit teratology study be submitted in which dosing of the does occurs only from gestational days 6-18, with their sacrifice on day 29.

RAT CHRONIC/ONCOGENIC STUDY (Life Science Research # 83/KCI045/248 and Amendment # 84/KCI045/579)

This study was previously submitted by the registrant under Report No. 83/KCI045/248, dated 9/7/84, EPA Accession Nos. 255042 thru 255046 and was reviewed by the Agency (Q. Bui memo of 2/15/85). In this memo, the study was classified as Core Supplementary Data pending the submission of additional information and/or clarification relative to:

1. Results of the test chemical analyses performed at different intervals throughout the study.
2. "Final" histopathologic reports
3. Identical cholinesterase activity values noted for different animals.
4. Significant differences in cholinesterase activity values noted between males and females of the same group.
5. Sensitivity of the test methods used to determine urinary protein.
6. Historical control data for testicular interstitial cell adenomas and pancreatic islet cell adenomas.

An addendum to the rat chronic/oncogenic study was submitted by the registrant under Report No. 84/KCI045/579, dated 10/18/84, EPA Accession Nos. 256011 thru 256016 and was reviewed by the Agency (Q. Bui memo of 7/12/85). It was recommended that this study remain classified as Core Supplementary Data. However, issues # 1 and 2 were adequately addressed by the registrant.

In this action (EPA Accession No. 258188, submitted to the Agency on 6/7/85), the registrant provided additional information and/or comments to the remaining four issues (#3 thru 6)

Issue #3: Identical Cholinesterase Values Noted for Different Animals

Agency's Position:

"Identical cholinesterase activity values were reported for different animals in the study".

Registrant's Comments:

"Cholinesterase activity is measured by autoanalyser. The amount of activity is indicated by the degree of deflection of a pen trace on a moving chart. The chart is divided into 100 segments, and the deflection is measured to 0.5 of a segment. A standard of known activity is analysed, and a factor is calculated from the relationship between the known activity and the degree of deflection. The amount of activity in the sample is then estimated by multiplying the deflection obtained with the sample by the calculated factor". The registrant concluded that, in the absence of a compound-induced effect, "cholinesterase activity would be, in general, comparable".

Agency's Response:

The cholinestarase activity as determined in this study was carried out by autoanalyser based upon the method of Humiston and Wright (1967). The cholinesterase activity values from the samples were calculated by multiplying the tracing deflection of each sample by a constant factor generated from a standard.

Therefore, due to the limitation of measurement discrimation and the low activity of plasma pseudocholinesterase levels, identical values may be obtained from different animals, particularly if the chemical tested has no cholinesterase inhibiting activity. A re-evaluation of the butyryl-cholinesterase levels suggested that apparently a factor of 20 was used.

The investigators' comments satisfactorily rectify this issue.

Issue #4: Significant Differences in Cholinesterase Activity Values Between Sexes

Agency's Position:

"Unusual, significant differences in cholinesterase activity values were reported between males and females of the same group. At all intervals studied, the plasma butyryl ChE and acetyl ChE activities of females were always 2-4 fold higher than those of males. No explanation was given by the investigators".

Registrant's Comment:

"The marked sex-difference in blood cholinesterase activities observed in this study are consistent with our experience at Life Science Research". The registrant also listed the mean control values collected from a rat chronic study and referred to some published references to substantiate their comments.

Agency's Response:

The newly submitted control values for plasma acetyl and butyryl cholinesterase activity indicate a 4-5 fold difference between males and females. The historical control data compiled in 1984 from Hazleton Lab. also demonstrate 3-4 fold differences between males and females, with females always having higher plasma cholinesterase activity. Cholinesterase data from Chevron Company reveal that the average values are 2.9 times greater in females.

These data collectively suggest that a substantial sex difference in plasma cholinesterase activity may exist between untreated males and females.

The registrant has satisfactorily rectified this issue.

Issue #5: Sensitivity of test methods used to determine urinary protein concentrations.

Agency's Position:

"No compound-related effects on the urinary protein concentration were evident from the reported data. However, Table 11E on page 141 erroneously indicated a value of 1050 mg/dl for the control males at week 103. Based upon the raw data, this value should be corrected to 1000 mg/dl" and "no explanation was given by the investigators as to the sensitivity of the test method used. Apparently, 1000 mg/dl was the maximum level detectable by this method".

Registrant's Comment:

The registrant provided a description of the method used and indicated that 1000 mg/dl was the upper limit of detection. Furthermore, the testing facility acknowledged that the value given for control males at week 103 was a typographical error.

Agency's Response:

This reviewer concurs with the registrant and testing facility.

Issue #6: Historical Control Data for Neoplastic Findings

Agency's Position:

" Increases in testicular interstitial cell adenomas and pancreatic islet cell adenomas were found in this study. Historical control data for these neoplasms collected by the testing facility for this species and strain must be submitted".

Registrant's Comment:

Historical control data collected from the testing facility for testicular cell adenomas and pancreatic islet cell adenomas are submitted. The registrant also included seven references pertaining to these findings in F-344 rats.

Agency's Response:

1. Pancreatic Islet Cell Adenomas

Pancreatic cell adenomas were found at a higher incidence in the treated males. The numbers of animals affected were 1/59 (1.6%), 3/60 (5.0%), 10/59 (16.9%), and 3/59 (5.1%) for the groups receiving 0, 20, 100, and 500 ppm, respectively. Although statistical differences were found only for the 100 ppm group, analysis of the data showed a positive trend on dose (P=0.041). It should be stressed that the most appropriate and important comparison of a treated group is with the concurrent control. However, in cases the comparison to the concurrent control may lead to equivocal results, additional historical control data are needed to make a clear and concise interpretation of the results, hence the Agency's request. The newly submitted historical control data indicated that pancreatic islet cell adenomas were found in 41/372 males (11.0%) in studies conducted at the same testing facility (Life Science Res.) . Incidences of 6.2% (Coleman et al., 1977), 3.5% (Goodman et al., 1979), 4.9% (Tarone et al., 1981), and 21.7% (Solleveld et al., 1984) were documented in the literature. These data suggest that pancreatic islet cell adenomas in male F-344 rats vary considerably and apparently are related to aging of the animals.

It is concluded that the incidence of pancreatic islet cell adenomas noted in Bolero treated males apparently was not compound-related due to:

a) Lack of dose response relationship.
b) Incidences found in treated males are comparable to those of the historical control data and of the published literature.

2. Testicular Interstitial Cell Adenomas

These adenomas were observed in 76.6%, 85.0%, 88.3%, and 90.0% of males in the groups receiving 0, 20, 100, and 500 ppm, respectively. Statistical differences from controls were noted at the 500 ppm dosage level but analysis of the data did not indicate a positive trend increase. The testing facility has provided an overall historical incidence of 96% in terminally sacrificed

animals (106 - 121 weeks). The overall incidences of this neoplasm in historical control animals which "died on study" ranged from 72 - 92%. The incidences of this neoplasm reported in the literature for F-344 rats were 86% (Sass et al. 1975), 88% (Coleman et al., 1977), 80.5% (Goodman et al., 1979), 86% (Sanford et al., 1982), and 95.6% (Solleveld et al., 1984). Therefore, although the incidence in the 500 ppm group (90%) was statistically significantly different from the concurrent control (76.6%), it was not different from the historical control data and published references. Consequently there is not enough evidence to indicate that the increase in testicular interstitial cell adenomas found in the 500 ppm group was compound-related. This increase apparently may be related to group survival differences since survival in Bolero-treated males was greater than in controls.

DISCUSSION AND RECOMMENDATION

Re-evaluation of the recently submitted data provided the following conclusions:

1. There is no conclusive evidence to indicate that the findings of testicular interstitial cell adenomas and pancreatic cell adenomas in the treated males were compound-related

2. The registrant has satisfactorily rectified all issues addressed in the Agency's review of 2/15/85.

3. The rat chronic/oncogenic study with Technical Bolero (Life Science Res.) is upgraded to Core Minimum Data with the following findings:

Systemic NOEL = 20 ppm (lowest dose tested)
Systemic LEL = 100 ppm (decreased food consumption, decreased body weight gain, increased blood urea nitrogen)
Carcinogenic potential negative up to and including the highest dosage level tested (500 ppm).

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology, rabbit Bio/dynamics Inc., # S-2241 12/20/83	Technical	258189	Maternal NOEL: Not determined Developmental tox. NOEL: Not determined Dose Levels: 0, 2, 20, and 100 mg/kg by gavage from days 7-29 of gestation		Supplementary
2-year feeding, rat Life Science # 83-KCI045/248 84-KCI045/579 7/83	Technical	258188	Systemic NOEL = 20 ppm (LDT) Systemic LEL = 100 ppm (decreased food consumption, body weight gain, increased blood urea nitrogen) Oncogenic potential negative up to and including a dose level of 500 ppm (HDT) Dose levels: 0, 20, 100, and 500 ppm in diet.		Supplementary upgraded to Minimum Data