UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: September 21, 1978

SUBJECT: Bonide Systemic Insecticide Contains Cygon EPA Registration#4-ELA Caswell#358 (Dimethoate)

FROM: William Dykstra, Ph.D
Toxicology Branch/HED (TS-769) WHD 9/21/78

TO: William Miller
Product Manager#16

Registrant: Bonide Chemical Co., Inc.
2 Wurz Avenue
Yorkville, N.Y. 13495

Recommendations:

1. Dimethoate is an RPAR Chemical.

2. In order to evaluate the human hazards from use the formulated product, the following toxicology studies are required to be submitted or referenced:

a. Oral LD_{50} - rats
b. Dermal LD_{50} - rabbits
c. Inhalation LC_{50} - rats
d. Primary Eye Irritation - rabbits
e. Primary Skin Irritation - rabbits
f. Skin Sensitization - Guinea Pigs

3. Teratologic evaluation of technical dimethoate is required.

4. The submitted toxicology studies may impact on the RPAR status of Dimethoate.

The studies are listed, summarized, and classified below:

a. Studies on the Chronic Oral Toxicity in Rats of Dimethoate Insecticide. The cholinesterase inhibition NOEL is 1.0 ppm of dimethoate in the diet of male and female Wistar rats for two years. Above this level brain, plasma and RBC cholinesterase inhibitions are observed. The absence of detailed tabular information on tumor type and incidence and the absence -of necropsy and histopathology tables precludes the establishment of oncogenic relationships and the histopathological NOEL for this study.

b. Mutagenicity testing of Technical Cygon Systemic Insecticide (Dimethoate) in the Ames Bacterial Test.

Negative results were obtained with five Salmonella typhimurium strains at doses up to 10, 000 µg in both plate and disc tests. Postive results were seen with Escherichia coli WP-2 UVRA- in the disc test. However, quantitation of results with this strain in the plate test revealed that the mutagenic potency of diemthoate is so low that it may be classified non-mutagenic by standard criteria. (continue on next page)

All tests were performed in the presence and absence of S-9 rat liver enzyme extract.

Classification: Core Minimum Data

c. Isodimethoate Mutagenicity testing; Negative results were obtained in TA100, Ta-1535, TA-1537 and WP-2UURA - with and without metabolic activation at 1000 µg per plate or disc using isodimethoate. Isodimethoate was toxic to TA-98 and TA-100 without metabolic activation.

Classification: Core Minimum Data

d. Bioassay of Dimethoate for Possible Carcinogenicity (NCI.) A bioassay of the carcinogenicity of the technical-grade dimethoate was conducted using Osborne-Mendel rats and B6C3F1 mice. Dimethoate is considered non-carcinogenic to these strains of rats and mice under the conditions of the experiment.

Classification: Core Minimum Data

*No RPAR criteria have been exceeded in these studies.

Product Name: Bonide Systemic Insecticide Contains, Cygon

Controls Houseflies and Insects and Mites on Ornamental Plants.

Ingredient	Percent Weight
Dimethoate (O,O-dimethyl S-(N-methyl carbamoylmethyl) phos-phorodithioate	23.4
[redacted]	
	100.0

Review:

Chemical Structure:

Submitted by American Cyanamid; Acc. No. 234501

I. Cygon 2E (EPA Refistration#241-82) Toxicology Book Three of Three, 7/28/78; submitted by American Cyanamid.

A. Studies on the chronic oral toxicity in rats of diemthoate insecticide (H.J. Lewerenz, G. Lewerenz, E. Engler, R. Plass German Academy of Sciences in Berlin; Central Institute of Nutrition; Dec. 1970)

300 Wistar rats were randomly divided into five groups of 30/sex/group and fed diets of 0, 0.1, 1.0, 10 and 75 ppm of dimethoate. The test period extended from September 1968 to September 1970.. Body weight and food consumption were monitored weekly during the first months of testing and monthly thereafter. Blood tests, administered in 12/sex/group or in the survivors of each group at 32, 50, 78 and 100 weeks, included: Hemoglobin, RBC, WBC, differential WBC and hematocrit (100 weeks only).

The cholinesterase activity of erythrocyte hemolysate and in plasma was measured for 6/sex/group after 1, 3, 12, 50, 75 and 100 weeks. After one and two years of testing, cholinesteraste activity in the cerebral homogenate was also determined by the micromethod of Meinecke and Oettel.

After one and two years of tesing the following clinical chemistries were determined on 6/sex/group: glucose, SGOT, SGPT. BUN, total protein, albumin, globulin.

After 1½ and two years of testing the following urinanalyses were determined on 6/sex/group: glucose, protein, SGOT, 24 hour amount, pH. The statistical analyses were made according to the *t*-test.

After one year of testing, 6/sex/group, and, at the end of the test, all surviving animals were killed by decapitation and tested macroscopically for abnormal changes. In the animals killed after one year, the weight of the brain, pituitary gland, thyroid gland, thymus, lung, heart, liver, spleen, kidneys, adrenal glands ~~and~~ were determined. The above-cited organs, with the exception of thymus, lungs and gonads, of the animals killed after two years were weighed. The brain, spinal medulla and peripheral nerve, thyroid, adrenal glands, thumus, spleen, mesenterial lymph nodes, stomach, duodenum, ileum, colon, liver, pancreas, gonads, kidneys, bladder, heart and lungs of 6/sex/group after one year were fixed in 8% neutral formulin for histological study. In addition, frozen bropsies of the liver were tainted with sudan for the detection of fat. The pituitary glands were fixed in susa. A histological study of these organs from 6/sex/group, with the exception of thymus and lung, also was undertaken after completion of the two-year feeding test.

Results: In the highest dose group, a significantly lower body weight was determined in weeks 2-20 in females and almost throughout the testing time in males. The amount of feed ingested by the test animals was the same as that of the control. During the major growth phase, the consumption of feed was calculated from the weight gain as related to feed intake. In the male group with the highest dimethoate concentration, there is a significantly lower consumption of feed than in the control group or in remaining test groups. Such a relationship was not determined for females.

Dimethoate concentrations up to 10 ppm were tolerated without externally detectable toxic symptoms. Females and males of the 75 ppm dimethoate group exhibited slight pilo-erection and exophthalmus initially and sometimes suffered a slight tremor. These symptoms disappeared after the fourth week of treatment. No toxic symptoms were noted during the remainder of the test in any groups fed with dimethoate.

A lower hemoglobin concentration was found among, the females of the 75 ppm group at 32 weeks, and a lower number of eosinophils was found in both sexes. In the 50th week of testing, no differences were found in hematologies of females but males in the 75 ppm test group again showed lower eosinophils. After 1½ years a larger number of erythrocytes was found in females at 75 ppm and an increase in leukocytes in the 75 ppm males.

After two years of testing no differences were found in the blood status of test animals.

The cholinesterase activity of the RBC hemolysate was reduced significantly throughout the test time in both sexes in the highest dose group, and up to the 75th week in females of the group with 10 ppm. While enzyme inhibition was present only in the highest dose group in plasma of the females, a reduction in plasma cholinesterase was seen in the males at 10 ppm.

Brain cholinesterase was inhibited in males and females at 10 and 75 ppm dimethoate after one and two years. The urine analyses and renal function tests indicated no substantial effects. After three months, the serum analyses yielded a lower concentration of urea-nitrogen in the 75 ppm males.

A lower weight of the ovaries was found in the females to which doses of 10 and 75 ppm were administered for one year. A lower weight of liver, spleen, adrenals glands and testes was found in 75 ppm males after one year. After two years, in females, the males of the 75 ppm group, a lower liver, kidney, adrenal weight was found.

The mortality of all groups was similar during the test period, despite a significant loss of animals after 1½ years due to an acute infection (gastroenteritis). Treating all animals with oxy tetracycline (three applications per oz 740 mg/kg body weight in three successive weeks) arrested the infection. Comparable numbers of male and female animals survived among the groups.

Although the report states that no correlation between the occurrence of tumors and dimethoate feeding exists, a detailed table of tumors type and incidence is not presented. Rather a table (Table 25) of total tumors of animals on test, macroscopically visible, is presented.

Similarly the report states that macroscopic and microscopic abnormal organ evaluation yielded no substance-linked alterations but detailed necropsy and histopathology tables are not presented. Rather a narrative of microscopic evaluation of organs examined is presented without regard to possible dose-related effects.

Conclusion: The absence of detailed information on tumor type and incidence and the absence of necropsy and histopathology tables firmly precludes the establishment or oncogenicity and the histopathological NOEL for this study. The cholinesterase inhibition NOEL is 1.0 ppm of dimethoate for two years. Above this level (10 and 75 ppm), brain, RBC and plasma cholinesterase inhibitions are observed.

Classification: Supplementary Data

(a) The registrant must submit detailed information on the type and incidence of tumors observed.
(b) The registrant must submit detailed necropsy and histopathology tables on the one year and two year sacrifices in order that the histopathological NOEL can be established.

B. Mutagenicity testing of technical Cygon Systemic Insecticide (Dimethoate/in the Ames Bacterial Test (ACCO, Project No. 0-796; 11/16/77)

Test Material: Cygon (dimethoate) Batch 12108

Compounds used as mutagenic standards:

(a) 2-aminofluorene (2-AF) is used as a mutagenic standard for TA-98 at 20 µg per plate. It requires activation by S-9 rat liver enzyme extract.

(b) N-methyl-N[1]-nitro-nitrosoguanidine (MNNG) is a potent produce of base-pair substitutions when used at 20 µg per plate. It is used for strains TA-1535, Ta-100 and E. Coli WP-2 UVRA-. This compound does not require activation by S-9 rat liver enzyme extract.

(c) 9-amino acridine(9-AA) is a mutagenic standard used at 50 µg per plate for TA-1537. It does not require activation by S-9 rat liver enzyme extract.

Methods and Results

1. Preparation of Rat Liver Enzyme Extract S-9.

Sprague-Dawley rats weighing approximately 200 gm were injected intraperitoneally with 100 mg of PCP Aroclor 1254. Five days later the rats were sacrificed, the livers removed aspectically and homogenized in 0.15 M KCl. The homogenate was centrifuged at 9000 Xg and the supernatant fluid was stored in 1.0 ml aliquots in liquid nitrogen. This extract was designated S-9 and its potency was tested by measuting the capacity of two-fold dilutions to convert the pro-mutagen 2-amino fluorene to a mutagen when tested with strain TA-98.

b. The plate test

The methods used were essentially those described by Ames et al. The test sample was tested at 10, 100 and 1000 µg per plate with and without metabolic activation in TA-98, TA-100, TA-1535, TA-1537 and WP-2 UVRA-. No increase in the number of revertants was seen with any of the five strains tested.

Control mutagenic compounds gave the appropriate expected results. The plate test was repeated using doses of 100, 1000 and 10,000 µg per plate for strains TA-98, TA-100, TA-1535, TA-1537 and TA-1538. (continue on next page)

Dimethoate was toxic at 10,000 µg per plate to TA-1537 in the absence of S-9 rat liver enzyme. No positive mutagenic responses were seen on any of the plates containing non-toxic doses.

In a third test, using duplicate plates at each dose level, doses of 10, 100, 1000, 5000 and 10,000 µg per plate were used for strains TA-98 and WP-2 UVRA⁻. Strain TA-98 showed no increase in the number of revertant colonies at any dose tested. Strain WP-2 UVRA⁻ displayed revertants at more than twice the number of spontaneous backround revertants at 1000, 5000, and 10,000 µg per plate.

The data for strain WP-2 UVRA⁻ were analyzed by least square linear regression to generate linear dose responses with and without S-9 rat liver enzyme extract. The mutagenic potencies using WP-2 UVRA⁻ were calculated to be 0.0063 revertants per nanomole in the presence of S-9 rat liver enzyme extract and 0.0078 in the absence of S-9 rat liver enzyme extract. Because the mutagenic potency is less than 0.01 revertants per nanomole in each case, dimethoate may be considered as a non-mutagen according to Ames et al.

c. The Disc Test

The test sample was tested at 1000 ug per disc with four strains (TA-98, TA-100, TA-1535 and WP-2 UVRA⁻). No increase in revertants was observed in the presence or absence of S-9 rat liver enzyme extract. The mutagenic control compounds gave the expected responses, indicating that the test system was capable of detecting mutagens and that the S-9 rat liver enzyme system was functional.

The disc test was repeated for five strains (TA-98, TA-100, TA-1535, TA-1537 and WP-2 UVRA⁻) using a dose of 10,000 µg per disc and the appropriate mutagenic control compounds. No increase in revertants was observed in the presence or absence of S-9 rat liver enzyme extract at non-toxic doses for Salmonella strains TA-98, TA-100, TA-1535, and TA-1537. E. Coli strain WP-2 UVRA⁻ did show some increase in revertants in the viscinity of the disc both in the presence and absence of S-9 rat liver enzyme extract.

Conclusion: Dimethoate gave a negative mutagenic response for each Salmonella typhimurium strain used. Strains TA-98, TA-100, TA-535, TA-1537 and TA-1538 were tested at various dose levels in Ames plate and disc tests. Each test was performed both with and without S-9 rat liver enzyme extract.

Dimethoate gave a positive response in the Ames disc test with and without S-9 rat liver enzyme extract at high doses when E. Coli strain WP-2 UVRA⁻ was used. When WP-2 UVRA⁻ was used in the Ames plate test, positive responses were obtained. The data which were obtained allowed quantitative calculation of mutagenic potency. The mutagenic potency of dimethoate was less than 0.01 revertants per nanomole. By standard methology of Ames et al, dimethoate may be classified as a non-mutagen.

Classification: Core Minimum Data

C. Mutagenicity testing of Isodimethoate (O,S-dimethyl S-methyl carbamoyl methyl ester, phosphorodithioic acid) (ACCO; Report No. M77-486; 9/27/77)

Test Material: Isodimethoate; CL number 35, 386

The disc test and plate test were used with TA-98, TA-100, TA-1535, TA-1537 and WP-2 UVRA⁻ with and without metabolic activation. Isodimethoate was tested at a level of 1000 µg per plate or disc.

Results: Non-mutagenic under test circumstances. Toxic to TA-98 and TA-100 at 1000 ug without S-9 metabolic activation.

Conclusion: Isodimethoate is non-mutagenic at 1000 µg per plate or disk.

Classification: Core Minimum Data

D. Bioassay of Dimethoate for possible carcinogenicity (NCI-CG-TR-4, Jan. 1977)

Test Material: Technical dimethoate (94-96%)

A bioassay of the carcinogenicity of the technical-grade dimethoate was conducted using Osborne-Mendel rats and B6 C3F1 mice. The test material was administered in feed to groups of rats (50/sex/group) at the MTD and ½ MTD (500 and 250 ppm) for 80 weeks, followed by 35 weeks of Observation. Initial doses were not well tolerated; therefore, they were reduced during the study. The "time-weighted average doses" for male rats were 155 and 310 ppm; for female rats 192 and 384 ppm. All surviving rats were killed between 113 and 115 weeks.

Dimethoate was administered in feed to groups of 50 male and 50 female mice at two concentrations (MTD and ½ MTD). Female mice and male mice received the same diet containing 250 and 500 ppm of dimethoate. Female mice received the diet for 80 weeks. However, high-dose males were returned to the control diet at 60 weeks and low-dose males at 69 weeks. All surviving mice were killed between weeks 93 and 94. Matched control animals consisted of 10/sex for rats and mice. All animals were observed for signs of toxicity twice daily and body weights were recorded at regular intervals until 110 wks. for rats and 90 weeks for mice. All animals were palpated for masses at each weighing. Those animals appearing moribund at the time of clinical examination were killed and necropsied. In the chronic study, the following 23 tissues and organs were taken from killed animals and where feasible, from animals found dead: brain, pituitary, lymph nodes (cervical and mesenteric), thyroid, parathyroid, salivary glands, lung, heart, diaphragm, stomach (pylorus and fundus), duodenum, jejunum or ileum, large intesting, pancreas, adrenal, kidney (longitudinal and transverse), liver, skin, entire gonads, urinary bladder, prostate or uterus, and femar with marrow. Tissues were preserved in 10% buffered formulin, sectioned routinely and stain with H & E.

Results: Tremors and hyperexcitability, both indications of dimethoate toxicity, were observed in the treated animals. Body weight data was comparable among control and test groups for both rats and mice.

Survival data was comparable between control and test groups for low dose rats and mice of both doses. At termination of the rat study there appeared to be a dose-related (high dose) mortality effect due to dimethoate, but this was obscured by the ususually large number of deaths in the male control rats.

Several non-neoplastic lesions occurred more frequently in test mice and rats than in controls and may have been related to exposure of the test material. It is considered that the low-dose group of rats and both dose groups of mice survived long enough to permit an evaluation of carcinogenicity.

Pathologic evaluation revealed no statistically significant increase in tumors associated with dimethoate treatment in either species of animal.

Conclusion: Dimethoate is considered non-carcinogenic under the conditions of the experiment.

Classification: Core-Minimum Data

TOX/HED:th:Reto Engler:9/8/78

10/11/78