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

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CASWELL FILE

8-7-86

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Submission of the Toxicology Branch Chapter for
Registration Standard for Diazinon.

TOX CHEM No. 342
TOX PROJECT NO. 23

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Attached is the Toxicology Branch (TB) Chapter for the
Registration Standard for diazinon including the following sub-
parts:

1. Diazinon Policy Discussions
2. Table A: Generic Data Requirements for Diazinon
3. Summary of the Evaluated Data ("one liners" and
selected detailed reviews).

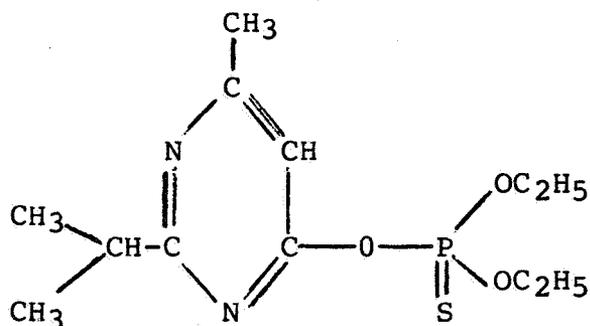
Diazinon Policy Discussions

A. Identification of the chemical and use summary.

Diazinon (see chemical structure below) is an organo-phosphate insecticide and nematocide which has a variety of agricultural and associated tolerances for residues and other uses including domestic applications. Many formulations containing diazinon are readily available at retail outlets.

The chemical structure and chemical name for diazinon are:

chemical structure:



chemical name: diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.

Commercially diazinon is also known as Basudin, Dazzel, Diagran, Diaterr-Fos, Diazajet, Diazatol, Diazide, Diazol, Dizinon, Dyzol, Dzn, Fezudin, Nipsan, Serolex, Spectracide, and Knox Out (a microencapsulated product). The principle manufacturer of diazinon used in the United States is the Ciba-Geigy Corporation.

B. Data Summary

1. One Liners

Attached

2. Policy Discussions of Diazinon Toxicity Problems

The toxicity data base for diazinon consists of a mixture of historical studies (studies circa 1950-1960 which do not have current criteria for investigational endpoints) and several contemporary studies. In addition, review of the published literature suggests that there are some potential or alleged special problems with this chemical. Several of the historical studies with diazinon will have to be repeated. Some special aspects will have to be included in conventional studies to address the alleged potential special problems with diazinon.

A. The data gaps for diazinon are identified as follows:

Delayed type neurotoxicity - hens
Subchronic feeding - rats
Subchronic feeding - dogs
21-day or 90-day dermal - rabbits
21-day or 90-day inhalation - rats
Chronic feeding - rats
Chronic feeding - dogs
Oncogenicity - rats
Multigeneration reproduction - rats (with special assessment of behavioral and endocrine development).
Mutagenicity - Selected study types and summary table of available studies (see below)
Metabolism and Pharmacokinetics

[Note: The technical diazinon used in all studies to satisfy the above data gaps must be identified with respect to percent purity. The percent (or ppm) content of all organophosphorus impurities must be identified. In particular the concentration of sulfotepp must be stated.]

B. Discussions of the Data Gaps.

1. Delayed type neurotoxicity. Since diazinon is an organophosphate insecticide it must be tested in hens for delayed type neurotoxicity. The two studies which attempt to assess this problem which are available to TB do not meet current

CORE MINIMUM criteria. An additional study will have to be submitted.

2. Subchronic Feeding Rats and Dogs. The available subchronic feeding studies in rats and dogs do not meet CORE MINIMUM criteria and usually only ChE activity (and a few other endpoints) were investigated. These subchronic studies are necessary to assist TB in assigning a NOEL for purposes of ADI setting. TB also recommends that subchronic studies in rats and dogs be conducted to assess the appropriate dose levels to be used for the chronic studies.
3. Chronic feeding in dogs and rats. New studies are critically needed to assist in determining the ADI (see Tolerance Re-assessment below). The historical studies examined only a limited number of investigational endpoints and used only a few specimens per dose group. The endpoint investigated in these studies was usually ChE inhibition. New chronic feeding studies must establish a NOEL for plasma, RBC and brain ChE inhibition as well as the other investigational endpoints recommended by the current EPA Guidelines for chronic toxicity testing.
4. Oncogenicity testing.

In mice. The NCI study has been assigned CORE MINIMUM status. There was no evidence of an oncogenic response to diazinon in this study.

A second study with mice conducted at Industrial Biotest (IBT) has been determined to be INVALID (refer to the report from the Dynamac Company following page R-38).

In rats. The NCI study could not be accepted as a definitive oncogenicity study in rats. The chronic feeding study requested above should also include a sufficient number of rats to be a combined chronic feeding/oncogenicity study.

5. Teratology and reproduction.

Teratology. Review of the published literature suggests that diazinon (as well as other organophosphates) may be potentially teratogenic because injecting these chemicals directly into developing avian eggs results in chicks with structural malformations. The relevance of these findings, however, to mammals is questionable. The registrant has submitted teratology studies deemed to be CORE MINIMUM or better for both rats and rabbits and has thus met the data requirements for teratology testing. None of the mammalian studies which have been reviewed indicated that diazinon causes structural abnormalities or shows other signs of developmental toxicity. Thus, diazinon is not considered a teratogen in mammals by TB. The apparent positive response noted in avian eggs may relate to the mode of direct admin-

istration, a condition that would not resemble human exposure resulting from the use of diazinon.

Published articles also indicate that diazinon may have effects on the endocrine system (i.e. changes in hepatic metabolism of corticosterone and in plasma levels of this hormone, see Spyker et al in J. Environ. Pathology and Toxicology 2: 357-369, 1978) and on the motor function of pups born of dams dosed with diazinon during gestation. At least one study, however, could not repeat these reported observations as published (see p. R-23 of this Registration Standard).

The possibility that diazinon may be a behavioral teratogen as reported in the literature (Spyker and Avery in J. Toxicol. Environ. Health 3:989-1002, 1977) was deferred to Dr. William Sette of TB for review. Dr. Sette's conclusion (refer to memo from W.F. Sette to J. Doherty dated June 23, 1986) was that "this study provides no convincing evidence of an effect of diazinon on the nervous system".

Because TB already has CORE MINIMUM and higher data on teratology with diazinon, no additional teratology studies will be required to further investigate the alleged endocrine and behavioral effects of diazinon. These parameters should be investigated, however, as a part of the multigeneration reproduction study (see below).

Reproduction. There is no multigeneration reproduction study with diazinon which meets CORE MINIMUM criteria. A second study will have to be provided. This replacement study should include tests to assess the motor function and status of the endocrine systems (including blood levels of several hormones) of the pups for each generation. The registrant is requested to submit a protocol of the planned tests to assess both the behavioral and endocrine aspects of the multigeneration study.

6. 21-day dermal and inhalation studies.

These requirements relate to the many uses of diazinon (both domestic and non-domestic) which result in repeated dermal and respiratory exposure. The studies must demonstrate NOELs for plasma, RBC and brain ChE as well as investigate the usual endpoints recommended by EPA's guidelines.

7. Mutagenicity.

The following study types need to be submitted:

- i. in vitro mammalian gene mutation studies (i.e. mouse lymphoma L5178Y and Chinese hamster type assays)
- ii. study assessing the potential effects for structural chromosomal aberrations
- iii. studies assessing the potential for inducing sister chromatid exchanges in both in vivo and in vitro systems.

This requirement relates to the inadequate in vivo study (Mutation Research 118:61-68, 1983 see review on page R-51) that reported diazinon at very low concentrations induces sister chromatid exchanges in mudminnows. TB requests that the problem be addressed by assessing the potential for diazinon to induce this chromosome effect in both in vivo and in vitro study systems. One of the in vivo studies should repeat this assay in the same species of mudminnows used in the original report as well as in intact mammals. In vitro assays should be performed both with and without mammalian metabolic activation.

The registrant is also requested to provide a summary table which includes all available studies testing for mutagenic effects of diazinon.

Note: The reviews of the mutagenicity studies were secondarily reviewed by Dr. Irving Mauer of TB and the recommendations for additional testing were also made in collaboration with Dr. Mauer.

8. Metabolism and pharmacokinetics.

Although several studies from the published literature were available for review, there were no studies with supporting data together with methods and procedures. Metabolism and pharmacokinetic studies in rats need to be submitted. Particular attention to identification of the metabolites should be included.

9. Antidote data.

Diazinon is an anticholinesterase organophosphate insecticide and atropine is antidotal to toxicity for this class of insecticides. TB, however, requests that all information that is available on the effectiveness of atropine and 2-PAM as antidotes to diazinon intoxication be submitted to the Agency. In particular, information on the dose levels of these two agents given together or in combination necessary to be effective is especially desired.

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C. Risk Assessment/Tolerance Reassessment

1. Risk Assessment

Reviews of the available studies with diazinon have not indicated either an oncogenic or other response which would require a statistical risk assessment.

2. Tolerance Reassessment

There are numerous tolerances established for diazinon for many RACS ranging from 0.1 ppm to as high as 60 ppm (on grass for forage) and some 100 or more commodities are included. Refer to the CFR 180.153 (July 1, 1983) attached.

Historically the Acceptable Daily Intake (ADI) for diazinon was determined using a dog subacute dosing study (of 31 days duration) with a NOEL of 0.02 mg/kg/day based on plasma ChE inhibition and a safety factor of 10. Using this combination and taking into consideration published tolerances only, the percent ADI used up is 351% (refer to the computer printout attached).

The study used in setting the historical ADI (refer to page R-14 of this Registration Standard) does not meet CORE MINIMUM criteria and it is of only 31 days duration.

The ADI for diazinon was reevaluated as of June, 1986 for the TB ongoing RfD/PADI project. The following NOEL and safety factor was recommended for use:

NOEL = 0.009 mg/kg/day based on plasma ChE inhibition noted in a subchronic rat feeding study. [Davies, D.B. and Holub, B.J., Toxicological Evaluation of Dietary Diazinon in the Rat, Arch. Environ. Contam. Toxicol. 9:637-650, 1980).

Safety Factor (or modifying factor) = 10 (this is considered appropriate because the endpoint was plasma ChE inhibition).

PADI = 0.0009 mg/kg/day.

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This PADI will be evaluated by the RfD/ADI Committee in the later part of 1986 or early 1987. Refer to page R-13 for review of the subchronic rat studies. It should be noted that the above rat subchronic study is classified as SUPPLEMENTARY data.

Since both the historical ADI and the PADI calculated using the rat subchronic study (which is in fact lower than the ADI calculated from the dog study) result in greater than 300% of the ADI being used up, no new tolerances should be granted without addressing the problem that the PADI is exceeded.

D. Use Classification and Special Labelling.

Use restrictions. Diazinon is considered by TB to be of moderate acute toxicity. For example the acute oral toxicity in rats for technical grade diazinon is Toxicity Category II and the acute dermal toxicity has been reported as either Toxicity Category II or III. The principle toxicity problems relate to inhibition of ChE and its subsequent effects. No oncogenic or teratogenic effects are currently recognized in mammals. Thus, the use classification of products containing diazinon should follow normal rules for restriction and nonrestriction for usage.

Special Labelling Dermal Sensitization Precautionary Statement. Diazinon was tested with humans for potential sensitization reactions and several of the volunteers showed a positive response which was confirmed by repetition (see p R-44). Therefore, products containing diazinon should have the following precautionary statement (or the equivalent):

"May cause contact sensitization following repeated contact with skin in susceptible individuals. Avoid repeated contact with skin. If sensitization reaction results consult a physician".

E. Special Problem of Contamination with Sulfotepp.

Historically diazinon formulations have also contained quantities of the impurity sulfotepp which is more acutely toxic than diazinon itself. TB understands that the Ciba-Geigy Corporation presently uses stabilizing additives or otherwise limits diazinon formulations to a minimal level of sulfotepp such that the small amount of sulfotepp present does not significantly affect the toxicity of the product. Should product chemistry or Residue Chemistry Branch deferrals indicate that significant quantities of sulfotepp will be present, then TB may require specific toxicity studies with this contaminant.

§ 180.151

Commodity	Parts per million
Pears	3
Peas, shelled	15
Peas, unshelled	15
Peas, vine, with pod	15
Peas, vine, without pod	15
Pecans	0.1
Pineapples	0.1
Plums	1
Potatoes	10
Poultry, (excluding kidney)	3
Poultry, kidney	9
Sheep, mby	0.2
Sheep, meat	0.2
Sorghum	1
Sorghum, forage	5
Soybeans	1
Soybeans, straw	1
Sugarcane	0.1
Tangerines	5
Walnuts	5

(b) Tolerances are established for residues of dalapon (2,2-dichloropropionic acid) resulting from application of dalapon sodium-magnesium salt mixtures to irrigation ditch banks in the western United States in or on the following raw agricultural commodities. Where tolerances are established at higher levels from other uses of dalapon on the subject crops, the higher tolerance applies also to residues from the irrigation ditch bank use.

Commodity	Parts per million
Avocados	0.2
Citrus fruits	0.2
Cottonseed	0.2
Cucurbits	0.5
Flaxseed	2.0
Fruits, pome	0.2
Fruits, small	0.2
Fruits, stone	0.2
Grain crops (exc wheat)	0.5
Grasses, forage	2
Hops	0.2
Legumes, forage	2
Nuts	0.2
Vegetables, fruiting	0.2
Vegetables, leafy	0.5
Vegetables, root crop	0.2
Vegetables, seed and pod	0.5
Wheat	2

[43 FR 22359, May 25, 1978]

§ 180.151 Ethylene oxide; tolerances for residues.

A tolerance of 50 parts per million is established for residues of the antimicrobial agent and insecticide ethylene oxide, when used as a postharvest fumigant in or on the following raw agricultural commodities: Black walnut meats, copra, whole spices.

Title 40—Protection of Environment

§ 180.152 Sodium dimethyldithiocarbamate; tolerance for residues.

A tolerance of 25 parts per million is established for residues of the fungicide sodium dimethyldithiocarbamate, calculated as zinc ethylenebisdithiocarbamate, in or on melons.

§ 180.153 O,O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate; tolerances for residues.

Tolerances are established for residues of the insecticide O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate in or on the following raw agricultural commodities:

Commodities	Parts per million
Alfalfa, fresh	40.0
Alfalfa, hay	10.0
Almonds	0.5
Almonds, hulls	3.0
Apples	0.5
Apricots	0.5
Bananas (NMT 0.1 ppm shall be present in the pulp after peel is removed)	0.2
Beans, forage	25.0
Beans, hay	10.0
Beans, guar	0.1
Beans, guar, forage	0.1
Beans, lima	0.5
Beans, snap	0.5
Beets, roots	0.75
Beets, sugar, roots	0.5
Beets, sugar, tops	10.0
Beets, tops	0.7
Birdsfoot trefoil	40.0
Birdsfoot trefoil, hay	10.0
Blackberries	0.5
Blueberries	0.5
Boysenberries	0.5
Broccoli	0.7
Brussels sprouts	0.7
Cabbage	0.7
Cabbage, Chinese	0.7
Carrots	0.75
Cattle, fat (pre-s appl)	0.7
Cattle, meat (fat basis) (pre-s appl)	0.7
Cattle, mby (fat basis) (pre-s appl)	0.7
Cauliflower	0.7
Celery	0.7
Cherries	0.75
Citrus	0.7
Clover (fresh)	40.0
Clover, hay	10.0
Coffee beans	0.2
Collards	0.7
Corn, forage	40.0
Corn (inc sweet k + CWHR)	0.7

Chapter I—En

Com

Cottonseed
Cowpeas
Cowpeas, forage
Cranberries
Cucumbers
Dandelions
Dewberries
Endive (escarole)
Figs
Filberts
Grapes
Grass (NMT 40 ppm s appl)
Grass, hay
Hops
Kale
Km fruit
Lespedeza
Lettuce
Loganberries
Melons
Mushrooms
Mustard greens
Nectaries
Olives
Onions
Parsley
Parsnips
Peaches
Peanuts
Peanuts, forage
Peanuts, hay
Peanuts, hulls
Pears
Peavine hay
Peavines
Peas with pods (de moving any shell pre
Pecans
Peppers
Pineapples
Pineapples, forage
Plums (fresh prunes)
Potatoes
Potatoes, sweet
Radishes
Raspberries
Rutabagas
Sheep, fat (pre-s appl)
Sheep, meal (fat basis)
Sheep, mby (fat basis)
Sorghum, forage
Sorghum, grain
Soybeans
Soybeans, forage
Spinach
Squash, summer
Squash, winter
Strawberries
Sugarcane
Swiss chard
Tomatoes
Turnips, roots
Turnips, tops
Walnuts
Watercress

(Sec. 408(d), 68 S

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48 FR 14896, Apr

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0.1
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0.75
0.5
10.0
0.7
40.0
10.0
0.5
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0.7
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Chapter I—Environmental Protection Agency

§ 180.154

Commodities	Parts per million
Cottonseed.....	0.2
Cowpeas.....	0.1
Cowpeas, forage.....	0.1
Cranberries.....	0.5
Cucumbers.....	0.75
Dandelions.....	0.7
Dewberries.....	0.5
Endive (escarote).....	0.7
Figs.....	0.5
Fiberts.....	0.5
Grapes.....	0.75
Grass (NMT 40 ppm shall remain 24 hours after appli).....	60.0
Grass, hay.....	10.0
Hops.....	0.75
Kale.....	0.7
Kwi fruit.....	0.75
Lespedeza.....	1.0
Lettuce.....	0.7
Loganberries.....	0.75
Melons.....	0.75
Mushrooms.....	0.7
Mustard greens.....	0.7
Nectannes.....	0.5
Olives.....	1.0
Onions.....	0.75
Parsley.....	0.75
Parsnips.....	0.5
Peaches.....	0.7
Peanuts.....	0.75
Peanuts, forage.....	40.0
Peanuts, hay.....	10.0
Peanuts, hulls.....	10.0
Pears.....	0.5
Peavine hay.....	10.0
Peavines.....	25.0
Peas with pods (determined on peas after removing any shell present when marketed).....	0.5
Pecans.....	0.5
Peppers.....	0.5
Pineapples.....	0.5
Pineapples, forage.....	40.0
Plums (fresh prunes).....	0.5
Potatoes.....	0.1
Potatoes, sweet.....	0.1
Radishes.....	0.5
Raspberries.....	0.5
Rutabegas.....	0.75
Sheep, fat (pre-s appli).....	0.7
Sheep, meat (fat basis) (pre-s appli).....	0.7
Sheep, mby (fat basis) (pre-s appli).....	0.7
Sorghum, forage.....	10.0
Sorghum, grain.....	0.75
Soybeans.....	0.1
Soybeans, forage.....	0.1
Spinach.....	0.7
Squash, summer.....	0.5
Squash, winter.....	0.75
Strawberries.....	0.5
Sugarcane.....	0.75
Swiss chard.....	0.7
Tomatoes.....	0.75
Turnips, roots.....	0.5
Turnips, tops.....	0.75
Walnuts.....	0.5
Watercress.....	0.7

§ 180.154 O,O-Dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] phosphorodithioate; tolerances for residues.

Tolerances for residues of the insecticide O-O-dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] phosphorodithioate in or on the following raw agricultural commodities:

Commodity	Parts per million
Alfalfa.....	2.0
Alfalfa, hay.....	5.0
Almonds.....	0.3
Almonds, hulls.....	10.3
Apples.....	2.0
Apricots.....	2.0
Artichokes.....	2.0
Barley, grain.....	0.2
Barley, straw.....	2.0
Beans (dry).....	0.3
Beans, snap.....	2.0
Birdfoot trefoil.....	2
Birdfoot trefoil hay.....	5
Blackberries.....	2.0
Blubberies.....	5.0
Boysenberries.....	2.0
Broccoli.....	2.0
Brussels sprouts.....	2.0
Cabbage.....	2.0
Cattle, fat.....	0.1
Cattle, mby.....	0.1
Cattle, meat.....	0.1
Cauliflower.....	2.0
Celery.....	2.0
Cherries.....	2.0
Citrus fruits.....	2.0
Clover.....	2.0
Clover, hay.....	5.0
Cottonseed.....	0.5
Crabapples.....	2.0
Cranberries.....	2.0
Cucumbers.....	2.0
Eggplants.....	0.3
Fiberts.....	0.3
Goats, fat.....	0.1
Goats, mby.....	0.1
Goats, meat.....	0.1
Gooseberries.....	5.0
Grapes.....	5.0
Grass, pasture (green).....	2.0
Grass, pasture, hay.....	5.0
Horses, fat.....	0.1
Horses, mby.....	0.1
Horses, meat.....	0.1
Kwi fruit.....	10.0
Loganberries.....	2.0
Melons (honeydew, muskmelons, cantaloups, watermelons, and other melons).....	2.0
Nectarines.....	2.0
Nuts, pistachio.....	0.3
Oats, grain.....	0.2
Oats, straw.....	2.0
Onions.....	2.0
Parsley, leaves.....	5
Parsley, roots.....	2
Peaches.....	2.0
Pears.....	2.0
Peas, black-eyed.....	0.3

(Sec. 408(d), 68 Stat. 514 (21 U.S.C. 346a(e)))

[47 FR 42738, Sept. 29, 1982, as amended at 48 FR 14896, Apr. 6, 1983]

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File last updated 4/10/84

ACCEPTABLE DAILY INTAKE DATA

(Historical)

Log	NOEL	S.F.	ADI	RPI
mg/kg	ppm		mg/kg/day	mg/day/60kg
0.020	0.80	10	0.0020	0.1200

Published Tolerances

CROP	Tolerance	Food Factor	mg/day/1.5kg
Olives (104)	1.000	0.06	0.00092
Apples (2)	0.750	2.53	0.02846
Apricots (3)	0.750	0.11	0.00126
Beans, snap (12)	0.750	0.98	0.01104
Beets (14)	0.750	0.17	0.00195
Beet greens (13)	0.750	0.43	0.00034
Blackberries (15)	0.750	0.03	0.00034
Blueberries (18)	0.750	0.03	0.00034
Boysenberries (17)	0.750	0.03	0.00034
Broccoli (19)	0.750	0.10	0.00115
Brussel Sprouts (20)	0.750	0.03	0.00034
Cabbage, sauerkraut (22)	0.750	0.74	0.00828
Carrots (24)	0.750	0.48	0.00540
Cauliflower (27)	0.750	0.07	0.00080
Celery (28)	0.750	0.29	0.00322
Cherries (30)	0.750	0.10	0.00115
Citrus fruits (33)	0.750	3.81	0.04288
Collards (37)	0.750	0.08	0.00092
Corn, all types (38)	0.750	2.51	0.02824
Cranberries (44)	0.750	0.03	0.00034
Cucumbers, inc pickl (46)	0.750	0.73	0.00816
Dewberries (52)	0.750	0.03	0.00034
Escarole/endive (56)	0.750	0.03	0.00034
Figs (57)	0.750	0.03	0.00034
Grapes, inc raisins (66)	0.750	0.49	0.00552
Hops (73)	0.750	0.03	0.00034
Kale (75)	0.750	0.03	0.00034
Lettuce (84)	0.750	1.31	0.01472
Beans, lima (11)	0.750	0.19	0.00214
Loganberries (86)	0.750	0.03	0.00034
Melons (92)	0.750	2.00	0.02253
Mustard Greens (93)	0.750	0.06	0.00069
Nectarines (100)	0.750	0.03	0.00034
Onions (105)	0.750	0.83	0.00931
Parsley (110)	0.750	0.03	0.00034
Parsnips (111)	0.750	0.03	0.00034
Peaches (114)	0.750	0.90	0.01012
Peanuts (115)	0.750	0.30	0.00402
Pears (116)	0.750	0.26	0.00287
Peas (117)	0.750	0.69	0.00782
Peppers (120)	0.750	0.12	0.00133
Pineapple (123)	0.750	0.30	0.00333
Plums, inc brunes (125)	0.750	0.13	0.00149
Radishes (133)	0.750	0.03	0.00034
Raspberries (135)	0.750	0.03	0.00034
Romaine (147)	0.750	0.03	0.00034

Spinach(150)	0.750	0.13	0.00057
Strawberries(152)	0.750	0.18	0.00207
Sugar, cane&beet(154)	0.7	3.54	0.04093
Summer Squash(155)	0.750	0.03	0.00034
Swiss Chard(158)	0.750	0.03	0.00034
Tomatoes(163)	0.750	2.87	0.03234
Turnips(165)	0.750	0.05	0.00057
Turnip Greens(166)	0.750	0.03	0.00034
Water Cress(168)	0.750	0.03	0.00034
Wintersquash(171)	0.750	0.03	0.00034
Cattle(20)	0.750	7.18	0.08083
Sheep(145)	0.750	0.19	0.00216
Almonds(1)	0.500	0.03	0.00023
Filberts(58)	0.500	0.03	0.00023
Pecans(118)	0.500	0.03	0.00023
Walnuts(167)	0.500	0.03	0.00023
Bananas(7)	0.100	1.42	0.00213
Coffee(36)	0.200	0.75	0.00224
Cottonseed (oil)(41)	0.200	0.15	0.00045
Potatoes(127)	0.100	5.43	0.00814
Soybeans (oil)(148)	0.100	0.92	0.00138
Sweet Potatoes(157)	0.100	0.40	0.00060
Mushrooms(97)	0.250	0.03	0.00011
Butabagas(139)	0.750	0.03	0.00034
Kiwi Fruit(204)	0.750	0.03	0.00034
Wheat(170)	0.050	10.36	0.00777
Chinese Cabbage(177)	0.700	0.03	0.00032

MPI 0.1200 mg/day/60kg TARC 0.4218 mg/day/1.5kg % ADI 351.46

Unpublished, tox Approved 4L2991

CROP	Tolerance	Food Factor	mg/day/1.5kg
Broccoli(19)	0.000	0.10	0.00000
Brussel Sprouts(20)	0.000	0.03	0.00000
Cabbage, sauerkraut(22)	0.000	0.74	0.00000
Chinese Cabbage(177)	0.000	0.03	0.00000
Cauliflower(27)	0.000	0.07	0.00000
Collards(37)	0.000	0.08	0.00000
Kale(75)	0.000	0.03	0.00000
Mustard Greens(95)	0.000	0.06	0.00000
Kohlrabi(76)	0.700	0.03	0.00032

MPI 0.1200 mg/day/60kg TARC 0.4221 mg/day/1.5kg % ADI 351.74

Current Action Section 18 [84-CA-36]

CROP	Tolerance	Food Factor	mg/day/1.5kg
Grapes, inc raisins(65)	1.250	0.49	0.00920
Blackberries(15)	1.250	0.03	0.00056
Boysenberries(17)	1.250	0.03	0.00056
Bewberries(52)	1.250	0.03	0.00056
Loyanberries(86)	1.250	0.03	0.00056
Raspberries(135)	1.250	0.03	0.00056

MPI 0.1200 mg/day/60kg TARC 0.4341 mg/day/1.5kg % ADI 361.75

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TOXICOLOGY BRANCH ADI PRINTOUT

Date: 08/07/86

Diazinon

PADI = 0.000900 mg/kg/day

Caswell #342

NOEL = 0.0090 mg/kg

Safety Factor = 10

CFR No. 180.153

LEL = 0.0000 mg/kg

Status: ADI NOT VERIFIED BY AGENCY RFD COMMITTEE. Generic issue of significance of ChE inhibition. ORD pending 5/31/85.

RESIDUE CONTRIBUTION OF PUBLISHED TOLERANCES

CROP	TOLERANCE (PPM)	PETITION NUMBER	FOOD FACTOR	MG/DAY
1 Almonds	0.500		0.03	0.000225000
2 Apples	0.750		2.53	0.028462500
3 Apricots	0.750		0.11	0.001237500
7 Bananas	0.100		1.42	0.002130000
11 Beans, lima	0.750		0.19	0.002137500
12 Beans, snap	0.750		0.98	0.011025000
14 Beets	0.750		0.17	0.001912500
15 Blackberries	0.750		0.03	0.000337500
17 Boysenberries	0.750		0.03	0.000337500
18 Blueberries	0.750		0.03	0.000337500
19 Broccoli	0.700	4E2991]	0.10	0.001050000
20 Brussel sprouts	0.700	4E2991	0.03	0.000315000
22 Cabbage, sauerkraut	0.700	4E2991	0.74	0.007770000
24 Carrots	0.750		0.48	0.005400000
6 Cattle	0.750		7.18	0.080775000
27 Cauliflower	0.700	4E2991	0.07	0.000735000
28 Celery	0.750		0.29	0.003262500
30 Cherries	0.750		0.10	0.001125000
32 Chicory	0.700		0.03	0.000315000
33 Citrus fruits	0.750		3.81	0.042862500
36 Coffee	0.200		0.75	0.002250000
37 Collards	0.700	4E2991	0.08	0.000840000
38 Corn, all types	0.750		2.51	0.028237500
41 Cottonseed (oil)	0.200		0.15	0.000450000
44 Cranberries	0.750		0.03	0.000337500
46 Cucumbers, including pickles	0.750		0.73	0.008212500
52 Dewberries	0.750		0.03	0.000337500
56 Escarole/endive	0.750		0.03	0.000337500
57 Figs	0.750		0.03	0.000337500
58 Filberts	0.500		0.03	0.000225000
66 Grapes, including raisins	0.750		0.49	0.005512500
73 Hops	0.750		0.03	0.000337500
75 Kale	0.700	4E2991	0.03	0.000315000
84 Lettuce	0.750		1.31	0.014737500
86 Loganberries	0.750		0.03	0.000337500
92 Melons	0.750		2.00	0.022500000
97 Mushrooms	0.250		0.03	0.000112500
99 Mustard greens	0.750		0.06	0.000675000

RESIDUE CONTRIBUTION OF PUBLISHED TOLERANCES

CROP	TOLERANCE (PPM)	PETITION NUMBER	FOOD FACTOR	MG/DAY
100 Nectarines	0.750		0.03	0.000337500
104 Olives	1.000		0.06	0.000900000
105 Onions	0.750		0.83	0.009337500
110 Parsley	0.750		0.03	0.000337500
111 Parsnips	0.750		0.03	0.000337500
114 Peaches	0.750		0.90	0.010125000
115 Peanuts	0.750		0.36	0.004050000
116 Pears	0.750		0.26	0.002925000
117 Peas	0.750		0.69	0.007762500
118 Pecans	0.500		0.03	0.000225000
120 Peppers	0.750		0.12	0.001350000
123 Pineapple	0.750		0.30	0.003375000
125 Plums, including prunes	0.750		0.13	0.001462500
127 Potatoes	0.100		5.43	0.008145000
133 Radishes	0.750		0.03	0.000337500
135 Raspberries	0.750		0.03	0.000337500
139 Rutabagas	0.750		0.03	0.000337500
145 Sheep	0.750		0.19	0.002137500
147 Sorghum	0.750		0.03	0.000337500
148 Soybeans (oil)	0.100		0.92	0.001380000
150 Spinach	0.750		0.05	0.000562500
152 Strawberries	0.750		0.18	0.002025000
154 Sugar, cane and beet	0.750		3.64	0.040950000
155 Summer squash	0.750		0.03	0.000337500
157 Sweet potatoes	0.100		0.40	0.000600000
158 Swiss chard	0.750		0.03	0.000337500
163 Tomatoes	0.750		2.87	0.032287500
165 Turnips	0.750		0.05	0.000562500
166 Turnip greens	0.750		0.03	0.000337500
167 Walnuts	0.500		0.03	0.000225000
168 Water cress	0.750		0.03	0.000337500
170 Wheat	0.050		10.36	0.007770000
171 Winter squash	0.750		0.03	0.000337500
177 Chinese cabbage	0.700	4E2991	0.03	0.000315000
204 Kiwi fruit	0.750		0.03	0.000337500

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TMRC
0.007012 mg/kg/day (60kg BW, 1.5kg diet)

%PADI
779.083333

RESIDUE CONTRIBUTION OF TOX-APPROVED TOLERANCES

CROP	TOLERANCE (PPM)	PETITION NUMBER	FOOD FACTOR	MG/DAY
76 Kohlrabi	0.700	4E2991	0.03	0.000315000

TMRC
0.007017 mg/kg/day (60kg BW, 1.5kg diet)

%PADI
779.666667

RESIDUE CONTRIBUTION OF NEW (PENDING) TOLERANCES

CROP	TOLERANCE (PPM)	PETITION NUMBER	FOOD FACTOR	MG/DAY
214	0.750	6E3347	0.03	0.000337500

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TMRC
0.007023 mg/kg/day (60kg BW, 1.5kg diet)

%PADI
780.291667

Table A
Generic Data Requirements for Diazinon

Data Requirement	Composition	Use Patterns	Does EPA Have Data to Satisfy This Requirement? (Yes, No or Partially)	MRID No.	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)7
§158.135 Toxicology					
<u>Acute Testing:</u>					
81-1 - Oral LD ₅₀ -Rat	TGAI	All	Yes	146179,75927, 5002272,146179, 49330	No
81-2 - Dermal LD ₅₀ - Rabbit	TGAI	All	Yes	49330,146180	No
81-3 - Inhalation LC ₅₀ - Rat	TGAI	All	Yes	109043	No
81-6 - Dermal Sensitization	TGAI	All	Yes	--	No
81-7 - Acute Delayed Neurotoxicity - Hen	TGAI	All	Partial	55409,32142, 135228,125998, 135229	Yes ¹ /
<u>Subchronic Testing:</u>					
82-1 - 90-Day Feeding - Rodent - Nonrodent	TGAI TGAI	All All	Partial Partial	57233 57233	Yes ² / Yes ² /
82-2 - 21-Day Dermal ³ /-Rabbit	TGAI	All*	No	--	Yes ³ /
82-3 - 90-Day Dermal ³ /-Rabbit	TGAI	All*	No	--	Yes ³ /
82-4 - 21- or 90-Day Inhalation ³ /-rat	TGAI	All*	No	--	Yes ³ /
82-5 - 90-Day Neurotoxicity-Hen	TGAI	All	Partial	55412 50771,57033, 100895,52735,	No ⁷ /

*All uses resulting in repeated dermal or inhalation exposure.

Table A
Generic Data Requirements for Diazinon

Data Requirement	Composition	Use Patterns	Does EPA Have Data to Satisfy This Requirement? (Yes, No or Partially)	MRID No.	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)7
§158.135 Toxicology (cont'd)					
<u>Chronic Testing:</u>					
83-1 - Chronic Toxicity - Rat	TGAI	A,C,E	Partial	75932	Yes ^{4/}
Chronic Toxicity - Dog	TGAI	A,C,E	Partial	---	Yes ^{4/}
83-2 - Oncogenicity - Rat	TGAI	A,C,E	Partial	73372	Yes ^{4/}
Oncogenicity - Mouse	TGAI	A,C,E	Yes	73372,50291	No
83-3 - Teratology - 1st species (Rat)	TGAI	All	Yes	109033,131150	No
Teratology - 2nd species (Rabbit)	TGAI	All	Yes	79013	No
83-4 - Reproduction - 2 Generations	TGAI	A,C,E	Partial	55407	Yes ^{5/}
<u>Mutagenicity Testing:</u>					
84-2 - Gene Mutation	TGAI	All	Yes	132952	No
84-3 - Chromosome Aberration	TGAI	All	No	---	Yes
84-2 - Other Mechanism of Mutagenesis Sister Chromatid Exchange	TGAI	All	Partial	139603	Yes ^{6/}
<u>Special Testing:</u> General Metabolism	PAI OF PAIRA	A,C,E	No	---	Yes

FOOTNOTES FOR TABLE A

Composition: TGAI = technical grade of the active ingredient; PAI=pure active ingredient; PAIRA= pure active ingredient, radio-labeled.
The use patterns are coded as follows: A = Terrestrial, food crop, B = Terrestrial, nonfood, C = Aquatic, food crop, D = Aquatic, nonfood, E = Greenhouse, food crop, F = Greenhouse, nonfood, H = Domestic, outdoor, I = indoor.

- 1/ The available studies do not meet CORE MINIMUM criteria. A definitive study must be submitted.
- 2/ New subchronic rat and dog studies must be submitted. These studies should be designed (in part) to determine the Maximum Tolerated Dose for the chronic feeding studies in rats and dogs.
- 3/ 21- or 90-day dermal and inhalation. If the exposure pattern warrants a 90-day study, a study of this duration will be required. Otherwise, a 21-day study is required.
- 4/ The available chronic feeding studies with rats and dogs do not meet CORE MINIMUM criteria.
- 5/ The available multigeneration reproduction study does not meet CORE MINIMUM criteria.
- 6/ One study has demonstrated that diazinon induces sister chromatid exchange in mudminnows. TB is requesting that this study be repeated and additional data using both in vivo and in vitro sister chromatid exchange be generated to further assess the potential for diazinon to induce sister chromatid exchange.
- 7/ Study will not be required unless acute neurotoxicity study is positive or equivocal.

Tox Chem No. 342 (Diazinon)

File Last Updated --

Current Date 8/7/86

Study/Lab/Study #/Date	Material	EPA Accession No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
Acute Oral LD ₅₀ - Rats; T.B. Gaines in Toxicol. Appl. Pharmacol.; 14:515-534(1969); MRID No. 49330 and Toxicol. Appl. Pharmacol. 2:88-99; (1960); MRID No. 5002272	Diazinon	--	LD ₅₀ =108 (96 to 122) mg/kg males 76 (66 to 87) mg/kg females in peanut oil. LD ₅₀ = 250 (231-270) mg/kg males = 285 (259-314) mg/kg females in peanut oil.	II	SUPPLEMENTARY
Acute Oral LD ₅₀ - Rats (male only); Hazleton, (No study No.); August 26, 1953; MRID No. 75927	Diazinon	--	LD ₅₀ =100 to 150 mg/kg for males only. Typical ChE symptoms but pathology of survivors (7 days) reveals possible lesions in lungs, spleen, testes, kidneys, and liver.	II	SUPPLEMENTARY
Acute Oral LD ₅₀ - Rats; Life Science Research Israel, Ltd.; (# MAK/063/DZL); August 20, 1984 MRID NO. 146179	Diazol Technical (Diazinon)	--	LD ₅₀ =775 (583 to 967) mg/kg males 499 (363 to 635) mg/kg females 618 (506 to 730) mg/kg combined Symptoms typical of an organo-phosphate AChE inhibition. No abnormalities in survivors.	II	GUIDELINES
Acute Oral LD ₅₀ - Mice (males only); Hazleton (no Study No.); August 26, 1953; MRID No. 75927	Diazinon	--	LD ₅₀ = 82 (71 to 95) mg/kg males Autopsy of survivors revealed pale livers, kidneys and atonic intestines.	II	SUPPLEMENTARY
Acute Dermal LD ₅₀ - Rats; T.B. Gaines in Toxicol. Appl. Pharmacol. 14:515-534 (1969); MRID No. 49330	Diazinon	--	LD ₅₀ =900 (740 to 1107) mg/kg males 455 (379 to 546) mg/kg females.	II	SUPPLEMENTARY

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Tox Chem No. 342 (Diazinon)

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Current Date 8/7/86

Study/Lab/Study #/Date	Material	EPA Accession No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
Acute Dermal LD ₅₀ - Rats; Ciba-Geigy - Ltd.; #Siss 1679; May 25, 1972; MRID No. 114078	Diazinon	228039	LD ₅₀ > 2150 mg/kg (no deaths or symptoms).	III	SUPPLEMENTARY
Acute Dermal LD ₅₀ - Rabbits; Life Science Research; Israel, Study No. (none); September 7, 1984; MRID No. 146180	Diazol	--	LD ₅₀ > 2000 mg/kg	III	MINIMUM
Acute Inhalation LC ₅₀ - Rats; Ciba-Geigy Labs.; #SISS 1679; April 25, 1972; MRID No. 109043	Technical Diazinon	228039	LC ₅₀ =3.50 (3.08 to 3.97) mg/l	III	MINIMUM
Primary Dermal Irritation - Rabbits; Life Sciences Research (Ltd.); Israel, # MAK/066/DZL; September 4, 1986 MRID No. 146182	Diazinon Technical	--	Not irritating	IV	GUIDELINES
Neurotoxicity - Hens; Woodward Research Corp. (no number; June 4, 1964; MRID Nos.: 55409, 32142, 135228, 125998, and 135229;	Diazinon Technical	90392	Not neurotoxic at doses up to and including 200 ppm for 22 days (HDT)	--	SUPPLEMENTARY

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Tox Chem No. 342 (Diazinon)

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Current Date 8/7/86

Study/Lab/Study #/Date	Material	EPA Accession No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL		TOX Category	CORE Grade/Doc. No.
			LD ₅₀	LC ₅₀		
Neurotoxicity - Hens; Communicable Disease Center (No number) As published in AMA Archives of Industrial Health; 13:326-330 (year?); MRID Nos.: 50771, 57033, 100895, 52735, 55412	Diazinon Technical	90392			--	SUPPLEMENTARY
Subchronic feeding - Rats; Hazleton MFL-1102; May 29, 1956 MRID No. 57233	Diazinon 25W	--	NOEL = 2.0 ppm LEL = 4.0 ppm (plasma ChE inhib- ition only).		--	SUPPLEMENTARY
Subchronic (Capsule) - Dogs; Hazleton - MFL-1102; May 29, 1956 MRID No. 57233	Diazinon 25W	--	NOEL = 0.02 mg/kg/day LEL = 0.04 mg/kg/day (plasma ChE inhibition up to 35%).		--	SUPPLEMENTARY
Subchronic feeding - Rats; females (various times); Toxicol. Appl. Pharmacol.; 54:359-367 (1980); (Authors D.B. Davies and B.J. Holub); MRID No.	Diazinon	--	NOEL < 2 ppm for plasma ChE (20% inhibition in females)		--	SUPPLEMENTARY
Subchronic Feeding - Rats; females (various times); Arch. Environ. Contam.; Toxicol.; 9:637-650 (1980); (Authors D.B. Davies and B.J. Holub); MRID No.	Diazinon	--	NOEL = 0.1 ppm LEL = 0.5 ppm (plasma ChE inhibition)		--	SUPPLEMENTARY

Tox Chem No. 342 (Diazinon)File Last Updated ---Current Date 8/7/86

Study/Lab/Study #/Date	Material	EPA Accession No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL		TOX Category	CORE Grade/ Doc. No.
			NOEL	LEL		
Teratology - Rabbits; Science Applicators, Study No. 281005; July 28, 1981 MRID No. 79013	Diazinon	---	NOEL = 100 mg/kg (HDT) for teratology NOEL = 25 mg/kg maternal toxicity LEL = 100 mg/kg (lethality) NOEL = 100 mg/kg (HDT) for fetotoxicity Levels tested 0, 7, 25, and 100 mg/kg		---	MINIMUM
Teratology - Rats; Institute for Animal Reproduction; Study No. (none); November 1, 1979 MRID No. 131150	Diazinon	---	NOEL > 4 mg/kg (HDT) (No effects of diazinon noted although many investigational endpoints evaluated.)		---	SUPPLEMENTARY
Teratology - Rats; Ciba-Geigy; Report 52-83; April 19, 1985 MRID No.	Diazinon	257826	NOEL > 100 mg/kg (HDT) for teratogenic effects (none evident) NOEL = 20 mg/kg for maternal toxicity, LEL = 100 mg/kg (decreased food consumption and body weight gain) NOEL = 100 mg/kg (HDT) for fetotoxicity. Levels tested: 0, 10, 20, and 100 mg/kg		---	GUIDELINES
Teratology - Rat; Ciba-Geigy - Switz.; Study No. PH 2.632 May 16, 1974 MRID No. 109033	Diazinon	---	NOEL = 100 mg/kg/day (HDT) (apparently for maternal and fetotoxicity). Levels tested: 0, 15, 50, and 100 mg/kg		---	SUPPLEMENTARY

Tox Chem No. 342 (Diazinon)

File Last Updated --

Current Date 8/7/86

Study/Lab/Study #/Date	Material	EPA Accession No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, IEL	TOX Category	CORE Grade/ Doc. No.
3-Generation Reproduction-Rats; Woodward Research Corporation; May 20, 1965; MRID No. 55407	Diazinon	90392	From the original review by Dr. G. Whitmore (dated June 25, 1965): NOEL = 8 ppm (HDT) for 2 generations and 4 ppm (HDT) for 3 generations.	--	SUPPLEMENTARY
Chronic Feeding - Rats; Hazleton; December 22, 1955; MRID No. 75932	Diazinon 25W	90054	NOEL < 10 ppm (plasma and RBC ChE inhibition) At 100 ppm (brain ChE inhibition) At 1000 ppm (hematocrit depression (males)).	--	SUPPLEMENTARY
Oncogenicity - Rat; Gulf South Breeze Research Institute; NCI sponsored study Issued 79-1392 (NCI); 1979 MRID No. 73372	Diazinon	--	Not oncogenic at dose levels up to and including 800 ppm.	--	SUPPLEMENTARY
Oncogenicity - Mouse; Industrial Biotech #8580-09381; July 7, 1980; MRID No. 50291	Diazinon	--	Not oncogenic up to and including 100 ppm.	--	INVALID
Oncogenicity - Mouse; Gulf South Breeze Research Inst.; NCI Sponsored Study #79-1392; Issued 1979 MRID No. 73372	Diazinon	--	Not oncogenic up to and including 200 ppm (HDT).	--	MINIMUM
Chronic Feeding - Dogs (46 weeks max.); October 26, 1954	25% Formulation	90054	NOEL<4.6 mg/kg/day (ChE inhibition) [Study uses only 1 mongrel dog per sex per group.]	--	SUPPLEMENTARY

Tox Chem No. 342 (Diazinon)

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Current Date 8/7/86

Study/Lab/Study #/Date	Material	EPA Accession No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
Chronic Dosing - Monkeys; Woodward Research Corporation (No Study No.); June 1, 1966 (final report); July 21, 1964 (6-month report); January 29, 1965 (1-year report); MRID Nos. 57664, 64320, and 64319	Diazinon 50 WP	90737		--	SUPPLEMENTARY
Sensitization - Human; Hilltop Research Inst.; Studies Nos. M-2640, M-351, and 0-159; December 17, 1963 and June 25, 1964	Diazinon Technical and Diazinon 4E	232008	5 to 6 of 56 volunteers showed positive dermal sensitization reaction to Diazinon as technical or 4E. Observation confirmed by rechallenge.	--	ACCEPTABLE
Mutagenicity - Ames Test; SRI International; Contract #68-01-2458; October 1979; MRID No. 132952	Diazinon	--	No evidence of mutagenicity over the dose range of 0 to 5000 ug/plate in 5 strains of <u>S. typhimurium</u> with and without metabolic activation	--	ACCEPTABLE
Mutagenicity - Reverse Mutation in <u>E. coli</u> ; SRI International; Contract #68-01-2458; October 1979; MRID No. 132952	Diazinon	--	No evidence of mutagenicity over the dose ranges of 1 to 5000 ug/plate in <u>E. coli</u> WP2 <u>urvA</u> with and without metabolic activation.	--	ACCEPTABLE
Mutagenicity - Mitotic Recombination in <u>S. cerevisiae</u> D2; SRI International; Contract #68-01-2458; October 1979; MRID No. 132952	Diazinon	--	No evidence of repair over the dose range of 0.01 to 5.0 percent with and without metabolic activation.	--	ACCEPTABLE

Tox Chem No. 342 (Diazinon)File Last Updated ---Current Date 8/7/86

Study/Lab/Study #/Date	Material	EPA Accession No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, IEL	TOX Category	CORE Grade/ Doc. No.
Mutagenicity - DNA Repair in <u>E. coli</u> ; SRI International; Contract #68-01-2458; October 1979; MRID No. 132952	Diazinon	---	No evidence of repair over the dose range of 0.01 to 5 mg/disk.	---	ACCEPTABLE
Mutagenicity - DNA Repair in <u>B. subtilis</u> (H17/M45); SRI International; Contract #68-01-2458; October 1979; MRID No. 132952	Diazinon	---	No evidence of repair over the dose range of 0.01 to 50 mg/plate.	---	ACCEPTABLE
Mutagenicity - <u>Unscheduled DNA synthesis</u> ; SRI International; Contract #68-01-2458; October 1979; MRID No. 132952	Diazinon	---	No evidence of repair the dose range of 0.1 to 1000 <u>ug</u> /plate with and without metabolic activation.	---	ACCEPTABLE
Mutagenicity - <u>E. coli</u> Spot Test; Tierfarm AG, Switzerland; (No study number); November 9, 1970; MRID No.	Diazinon	---	No evidence of mutagenicity. Doses tested: 0.5, 5, and 50 percent. Tested only without activation in a disk spot test.	---	UNACCEPTABLE
Mutagenicity - Sister Chromatid Exchange - Chinese Hamster (V79) Cells; Roswell Park Memorial Institute in Mutation Research; 88:307-316 (1981); (Chen, Heueh, Siriami, and Huang) MRID No. 139603	Diazinon	---	Study report conclusion is that diazinon does not induce sister chromatid exchanges at all doses tested up to cytotoxic levels but not tested with metabolic activation.	---	UNACCEPTABLE

Tox Chem No. 342 (Diazinon)

File Last Updated ---

Current Date 8/7/86

Study/Lab/Study #/Date	Material	EPA Accession No.	LD ₅₀ , LC ₅₀ , PIS, NOEL, LEL	TOX Category	CORE Grade/Doc. No.
Mutagenicity - Sister Chromatid Exchange in Mudminnows; Mutation Research; 118:61-68 (1983); (Vigfusson, Wise, Persteiner, Dawson) MRID No.	Diazinon	--	Reported to be <u>positive</u> for inducing sister chromatid exchange in mudminnows at 5.4 x 10 ⁻¹⁰ and 5.4 x 10 ⁻⁹ M. Studies require validation and confirmation.	--	INCONCLUSIVE (inadequate but positive study)
Mutagenicity - Dominant Lethal (Mice) Ciba-Geigy - Switzerland; (No. 327507); March 20, 1975 MRID No.	Diazinon	--	No evidence of dominant lethal effect at either 15 or 45 mg/kg. Highest dose tested was not toxic and study report was deficient in describing procedures and data reporting.	--	UNACCEPTABLE

Study Type: Acute Oral LD₅₀ - (rats)

Accession No.: Not provided

MRID No.: 146179

Sponsor: Makhteshim Chemical Works, Ltd. (Israel)

Testing Laboratory: Life Science Research Israel Ltd.
(Study No. MAK/063/DZL)

Date: August 20, 1984

Review:

The test material for this study was described as "Diazol Tech Batch No. 660192-226" and was described as being a yellow liquid. The percent purity was not provided. The test material was dissolved in corn oil and the rats were dosed at a constant volume of 10 ml/kg. In the main study groups of 5 male and 5 female rats (Sprague-Dawley, CD) were dosed with either 250, 400, 640, or 1024 mg/kg and observed till death or fourteen days.

The following LD₅₀'s were determined:

775 (583 to 967) mg/kg for males.
499 (363 to 635) mg/kg for females.
618 (506 to 730) mg/kg for both sexes combined.

The principal reactions to treatment were decreased motor activity, diarrhea, ataxia, hunching, and proneness. Other signs were typical of organophosphate poisoning. The rats died on days 1 or 2 of treatment and were reported as being normal on day 3. The survivors showed expected body weight gains after abatement of the symptoms.

Necropsy: the animals which died showed distention and other symptoms of the gastrointestinal system. No abnormalities were found in the survivors.

This study is CORE GUIDELINES. A Quality Assurance Statement accompanies the report. Technical diazinon is Toxicity Category II (the female LD₅₀ is < 500 mg/kg).

Study Type: Acute Oral LD₅₀ - (rats)

Accession No.: Not provided

MRID No.: 49330 and 5002272

Sponsor: Communicable Disease Center

Testing Laboratory: Communicable Disease Center

Date: published in 1960 and 1969 (see below for citations)

Review:

The information was published in the following journals:

T.B. Gaines "The acute toxicity of pesticides in rats". Toxicology and Applied Pharmacology 2:88-99 (1960).

and

T.B. Gaines "Acute toxicity of pesticides". Toxicology and Applied Pharmacology 14:515-534 (1969).

In the first study, diazinon was one of 44 compounds tested for acute oral LD₅₀. Diazinon was dissolved in peanut oil and administered by stomach tube to both males and females. The number of dose levels tested and the number of rats of each sex dosed were not provided. Information on the onset and duration of the symptoms or necropsy observations were also not provided. The LD₅₀ was reported as being:

108 (96-122) mg/kg for males
76 (66-870) mg/kg for females

In the second study, diazinon was one of 100 compounds tested for acute oral LD₅₀. Diazinon was dissolved in peanut oil and administered by stomach tube to both males and females. The dose levels tested were not stated. Information on the onset and duration of the symptoms or necropsy were not provided. The LD₅₀ was reported as being (with 19/20 confidence limits):

250 (231-270) mg/kg for males
285 (259-314) mg/kg for females.

No explanation for the more than two fold difference in the LD₅₀s were provided. It is possible that the earlier study contained more impurities such as sulfotepp (TB speculation).

These studies are SUPPLEMENTARY. There is insufficient detail regarding dosing and the onset and duration of the symptoms.

Study Type: Acute Oral LD₅₀ - Mice and Rats

Accession No.: Not provided

MRID No.: 75927

Sponsor: Geigy Company Inc.

Testing Laboratory: Hazleton, (Study No. - not provided)

Date: August 26, 1953

Review:

Groups of 13 male mice were dosed with diazinon in corn oil by stomach tube. The symptoms resulting included squinting, depression, and diarrhea followed by tremors, lacrimation, and rapid respiration. Autopsy of the survivors revealed pale livers and kidneys and atonic intestines. Survivors lost weight. The LD₅₀ was determined to be 82 (71 to 95) mg/kg.

Groups of seven male albino rats were dosed with diazinon in corn oil by stomach tube. The symptoms resulting were depression, salivation, lacrimation, tremors, diarrhea, blood discharge and oily secretion. Survivors lost weight. Postmortem examination revealed hemorrhagic lungs and intestines, the intestines were also atonic and filled with a creamy material. Among the survivors, autopsy revealed pale and granular livers and kidneys, enlarged spleens with a roughed surface and some with irritation of the stomach. There was also noted hemorrhage of the testes in the rats dead in the 150 mg/kg test group.

This study is SUPPLEMENTARY. The LD₅₀ was estimated to be between 100 to 150 mg/kg for the male rat and 82 mg/kg for the male mouse. Toxicity Category II.

Study Type: Acute Dermal LD₅₀ - (Rats)

Accession No.: 228039

MRID No.: 114078

Sponsor: Ciba-Geigy

Testing Laboratory: Ciba-Geigy (No. SISS 1679)

Date: May 25, 1972

Review:

Rats (3 males and 3 females) were prepared by clipping and the test material applied at a dose level of 2150 mg/kg (no higher doses were reported as being possible).

No rats died. No symptoms of toxicity or local irritation were reported. No changes in the survivors were noted at autopsy after 7 days.

This study is SUPPLEMENTARY. Rats were used and only 3 per sex were tested. The data indicate that technical diazinon is Toxicity Category III by the dermal route.

Study Type: Acute Dermal LD₅₀ - (rats)

Accession No.: Not provided

MRID NO.: 49330

Sponsor: Communicable Disease Center

Testing Laboratory: Communicable Disease Center

Date: Published in 1960 (see below)

Review:

The information was obtained from the following citation:

T.B. Gaines, "The acute toxicity of pesticides to rats." Toxicology and Applied Pharmacology 2:88-99 (1960).

In this study diazinon was one of 44 compounds tested for acute dermal LD₅₀. The diazinon was dissolved in xylene prior to application. The dose levels and the number of rats dosed per dose level were not provided. The LD₅₀ with 19/20 confidence intervals was reported as:

900 (740-1107) mg/kg for males
455 (379-566) mg/kg for females

This information is SUPPLEMENTARY. There is insufficient detail regarding the onset and duration of the symptoms.

Study Type: Acute Dermal LD50 - (Rabbits)

Accession No.: Not provided

MRID No.: 146180

Sponsor: Makhteshim Chemical Works Ltd. (Israel)

Testing Laboratory: Life Science Research Israel, Ltd.

Date: September 7, 1984

Review:

A single group of five male and five female albino rabbits was dosed with 2000 mg/kg of "Diazol tech" (a yellow liquid) and observed for 14 to 15 days.

None of the rabbits died. No symptoms were reported and no gross necropsy lesions were noted.

This study is CORE MINIMUM. The technical "Diazol" is Toxicity Category III via the dermal route.

Study Type: Acute Inhalation LC₅₀ - (Rats)

Accession No.: 228039

MRID No.: 109043

Sponsor: Ciba-Geigy Ltd.

Testing Laboratory: Ciba-Geigy Toxicology Unit (No. S1SS 1679)

Date: April 25, 1972

Review:

Rats (9 males and 9 females per group) were exposed to aerosols (generated by a pressure nozzle) containing diazinon at each of three concentrations (1708, 3418, or 3680 mg/m³) for four hours and observed for signs of toxicity. It was stated that concentrations higher than the highest dose tested were not possible.

An LC₅₀ of 3500 (3080 to 3970) mg/m³ was determined. The rats died within 48 hours (except 2 which died sometime between 2 and 7 days. The symptoms were reported only as tachypnoea and apathy. Necropsy revealed hemorrhages and congested organs in the rats which died. No substance related necropsy findings were reported in the survivors. Analysis of the atmosphere revealed that most of the particles were less 7 microns in diameter as measured by Cascade impaction.

Conclusion: This study is CORE MINIMUM. The inhalation LC₅₀ of diazinon is established as being in Toxicity Category III.

Study Type: Primary Dermal Irritation (Rabbits)

Accession No.: Not provided

MRID No.: 146182

Sponsor: Makhteshim Chemical Works, Ltd. (Israel)

Testing Laboratory: Life Science Research Israel Ltd.
(No. MAK/066/DZL)

Date: September 4, 1984

Review:

Six young adult rabbits (2 males and 4 females) were prepared by clipping free of hair and the test material Diazol (Batch No. 660192-226) applied is a single dose of 0.5 ml impregnated on a gauze patch. The patch was held in place by a porous dressing strip which was secured by an adhesive bandage for 4 hours. The rabbits were observed for reactions at 1, 24, 49, and 72 hours after removal of the dressing.

No signs of local irritation at the site of application were reported. The irritation score was 0.

This study is CORE GUIDELINES. Technical diazinon is Toxicity Category IV. A signed Quality Assurance Statement accompanies the report.

Study Type: Neurotoxicity (Chickens, delayed type)

Accession No.: 90392

MRID No.: 55409 and 32142 (Study Report), 135228 (Addendum),
125998 (Protocol), 00135229 (Analysis of Tissues).

Sponsor: Geigy Corporation

Testing Laboratory: Woodward Research Corporation

Date: June 4, 1964

Review:

The following was taken from the original review of this study by George B. Whitmore, DVM dated June 25, 1965.

"Demyelination Study:

Groups of 10 hens each were exposed to 0, 2, 20, and 200 ppm diets of diazinon for approximately 50 days. TOCP at a 1000 ppm diet level was fed to 10 hens for positive controls.

Sections of brain, spinal cord, and peripheral nerve from the control and the 200 ppm Diazinon groups were negative for demyelination lesions. Paralysis was not observed in the controls nor in Diazinon fed birds. TOCP fed hens exhibited paralysis or an incoordinated gait by the third week of feeding. Degenerative changes of anterior horn motor neuronal cells were found in two TOCP fed hens.

This experiment demonstrated that a 200 ppm Diazinon diet for 50 days will not produce demyelination in hens."

The addendum to the report (prepared by Dr. M.T.I. Cronin, MRID No. 135228) states that the tissues were also prepared with Luxol Fast Blue stain for neural cells and fibers and also stained by the Marchi-Wolman technique for degenerating myelin. Neither of these stains revealed evidence that diazinon resulted in delayed type neurotoxicity under the conditions of this assay.

The results of the analysis of the tissues of the hens dosed with diazinon (MRID No. 00135229) indicated that the diazinon could not be detected in any of the tissues analyzed in fat, white and dark muscle, heart, kidney, liver, gizzard, and eggs.

Review of correspondence associated with the protocol for this study (MRID No. 125998) reveals that Dr. Whitmore advised the testor that no drug free period was included in the study and that asymptomatic hens would be examined. The study was apparently changed so that the hens were dosed for 22 days (not

50 as indicated) in Dr. Whitmore's review) and later sacrificed at day 50 (28 days without test material).

Conclusion: This study is CORE SUPPLEMENTARY. The procedure used, dosing continuously in the diet, is not an acceptable method for this study type. The hens should be dosed with a single dose near the LD₅₀ dose level for chickens (and protected with atropine if necessary) and observed for 21 days and a second dose at the LD₅₀ level administered at about 22 days.

Study Type: Neurotoxicity (Chickens-delayed type)

Accession No.: 90392

MRID No.: 50771, 57033, 100895, 52735, 55412

Sponsor: None - A Contribution from the U.S. Government

Testing Laboratory: Communicable Disease Center, Atlanta, Georgia

Date: (As published in AMA Archives of Industrial Health
13:326-330 Year?).

Review:

Diazinon was one of eight organophosphates tested in this experiment. The limited data presented indicated that diazinon (dose ranges 5 to 80 mg/kg) did not induce delayed type neuropathology following subcutaneous administration to groups of two chickens.

This study is SUPPLEMENTARY. The study is essentially a screening study that does not meet current standards for delayed type neurotoxicity testing. Subcutaneous injection is not an acceptable method of administration of the test material for this study type without justification.

[Note: This paper was authored by W.F. Durham, T.B. Gaines, and W.J. Hayes.]

Study Type: Subacute Feeding - Rats (varying times)

Accession No.:

MRID No.:

Sponsor: None

Testing Laboratory: Department of Nutrition, University
of Guelph

Date: 1980 [As published]

Review:

The following is the author's abstract as it appeared in the literature.

Comparative Subacute Toxicity of Dietary Diazinon in the Male and Female Rat (Toxicology and Applied Pharmacology 54, 359-367 [1980]; D.B. Davies and B.J. Holub).

Author's Abstract

"Male and female rats were fed semipurified diets containing either 2 or 25 ppm diazinon for varying times. At appropriate times animals were bled from the orbital sinus to facilitate measurement of plasma cholinesterase and erythrocyte acetylcholinesterase activities. Additional animals were sacrificed to enable determination of brain acetylcholinesterase activity. General nutritional evaluations included measurement of body weight gains and food consumption during the growing period. Feeding diazinon at the levels employed produced no visible toxic manifestations. Body weight gains and feed consumption were comparable among control and treated groups during all studies. Feeding 25 ppm diazinon for 30 days produced more significant reduction of cholinesterase activity in plasma (by 22-30%) and brain (by 5-9%) among treated females compared to corresponding males. Erythrocyte acetylcholinesterase activity was significantly more depressed (by 3-17%) in treated females relative to appropriate males at Days 21-28 of the feeding trial. At no time was cholinesterase activity in any tissue assayed more significantly reduced among treated males than females. Feeding 2 ppm diazinon for 7 days failed to modify erythrocyte acetylcholinesterase activity among both sexes relative to controls. Plasma cholinesterase activities of treated males were not significantly different from control values, whereas, treated females showed significant depression (by 20%) of plasma enzyme activity. This latter finding is of interest since 2 ppm is the "no effect" level of diazinon for the rat established by the FAO/WHO on the basis of studies which focused on the male sex. The results indicate the female rat to be more sensitive to the toxicity of dietary diazinon compared to the male."

This study is being classified as SUPPLEMENTARY data. Literature publications such as this can be validated only by reviewing the original data. This study and the following study indicate that rat plasma ChE may be inhibited at lower test dose levels than previously thought.

Study Type: Subacute/Subchronic (up to 92 days) Feeding - Rats

Accession No.:

MRID No.:

Sponsor: None

Testing Laboratory: Dept. of Nutrition, Guelph University

Date: 1980 (as published)

Review:

The following is the author's abstract as it was published.

Toxicological Evaluation of Dietary Diazinon in the Rat
(Arch. Environm. Contam. Toxicol. 9, 637-650 [1980];
D.B. Davies and B.J. Holub).

Author's Abstract

"Female Wistar rats were fed a semipurified diet containing either no pesticide or 0.1 to 15 ppm diazinon for up to 92 days. At specified times animals were bled from the orbital sinus to facilitate measurement of plasma and erythrocyte cholinesterase activity using a highly sensitive radiometric assay. Additional rats were sacrificed to determine brain acetylcholinesterase activity. General nutritional parameters measured included body weight gains and feed consumption during the growing period. Feeding diazinon at the levels employed produced no visible toxic manifestations. Treated animals showed weight gains and feed consumption which were comparable to appropriate controls. Feeding trials up to 90 days revealed that rats were highly sensitive to diazinon after 31 to 35 days exposure, as judged by reduction of plasma and erythrocyte cholinesterase activities. Brain acetylcholinesterase was judged to be insensitive to dietary diazinon (1.0 to 15 ppm), although moderate reduction (by 6%) of brain enzyme activity was noted among animals fed 10 ppm diazinon at Day 92. For all feeding trials, plasma cholinesterase was a more sensitive indicator of diazinon toxicity compared to erythrocyte or brain acetylcholinesterase. The "no effect" level of diazinon for the rat was judged to be 0.1 ppm in the diet, which translates into an equivalent daily intake of 9 ug/kg body weight/day. This "no effect" level is 20 to 50-fold lower than levels reported elsewhere in the literature, which may be attributed, in part, to the use of female animals in the present studies."

This study is being classified as SUPPLEMENTARY.

The following are comments on this study (and the previous study by the same authors) as provided by Dr. W. Dykstra in his original review of these papers (memorandum dated October 22, 1981).

Recommendations:

"The authors' judged "no effect" level for diazinon in female rats is 0.1 ppm (0.009 mg/kg/day). However, at the 0.5 ppm level of dietary exposure, the plasma cholinesterase doesn't decrease to 80% of the control values. Toxicology Branch has generally considered a decrease of 20% in cholinesterase activity to be within normal biological variation, even though the decreases in plasma cholinesterase at 0.5 ppm diazinon were statistically significant in comparison to the controls. This dietary level of 0.5 ppm resulting in less than 20% of plasma cholinesterase inhibition is judged to be the "no effect" level for diazinon. A "no effect" level of 0.5 ppm diazinon does not impact on the ADI based on the criteria used by Toxicology Branch."

Note: (Added by J. Doherty July 1986). During discussions between E. Budd, W. Dykstra, and J. Doherty in June of 1986, the ADI was reevaluated and this study showing a NOEL of 0.1 ppm was selected for use in setting the ADI. The graphical data (photocopied from the reprint) was used to show that plasma ChE (at 0.5 ppm) stays inhibited starting after the 7th day of treatment.

Discussion:

Table 6. Effect of feeding 0.1 to 2.0 ppm Diazinon on the activity of rat plasma cholinesterase*

Days on diet	Plasma cholinesterase activity ^b				
	Control	0.1 ppm	0.5 ppm	1.0 ppm	2.0 ppm
7	133 ± 6 ^c	124 ± 4 ^c	121 ± 7 ^c	109 ± 5 ^d	92 ± 4 ^e
14	167 ± 9 ^c	149 ± 6 ^c	141 ± 9 ^b	128 ± 7 ^c	107 ± 3 ^d
21	186 ± 7 ^c	178 ± 12 ^c	157 ± 12 ^b	140 ± 8 ^c	114 ± 4 ^d
28	195 ± 7 ^c	188 ± 8 ^c	168 ± 13 ^b	152 ± 9 ^c	118 ± 7 ^d
35	210 ± 8 ^c	203 ± 9 ^c	177 ± 14 ^b	152 ± 8 ^c	121 ± 6 ^d

* Each value represents the mean ± SE for 10 rats

^b Enzyme activities for each dietary group are expressed as a percentage of activity at Day 0. Activities at Day 0 for control, 0.1 ppm, 0.5 ppm, 1.0 ppm and 2.0 ppm groups were 0.47 ± 0.03, 0.48 ± 0.03, 0.47 ± 0.02, 0.49 ± 0.03, and 0.48 ± 0.02 μmole/ml plasma/min (Mean ± SE, n = 10), respectively

^{c,d,e} Values across each row having the same superscript letter(s) were not significantly different (p > 0.05) when tested by the method of Duncan

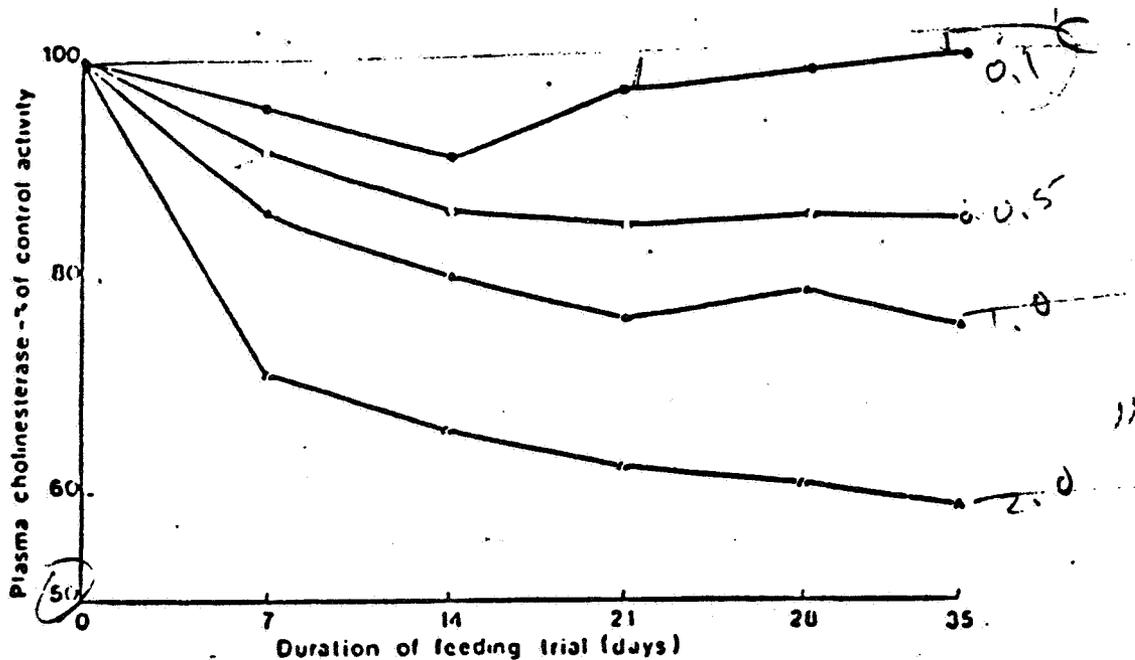


Fig. 4. Effect of feeding 0.1 to 2.0 ppm Diazinon on the activity of rat plasma cholinesterase. See also legend to Figure 1. 0.1 ppm (●), 0.5 ppm (△), 1.0 ppm (▽), 2.0 ppm (◇)

Study Type: Subchronic oral - rats

Accession No.: Not provided

MRID No.: 57233

Sponsor: Ciba-Geigy

Testing Laboratory: Hazleton Laboratories (No study number provided)

Date: May 29, 1956

Review:

[Note: Most of this study report is unreadable because of poor reproduction.]

Five groups of 15 male and female rats were dosed with diazinon (a 25W formulation of undefined composition was used) at dose levels of 0, 0.5, 1.0, 2.0, and 4.0 ppm (as active ingredient) for 28 or 29 days. There were no signs of reactions to treatment noted. The study maintains that the NOEL was 2 ppm. At 4 ppm plasma ChE was reported as being significantly inhibited. TB notes that the plasma ChE was also depressed at 2 ppm (14%) but this was apparently not significant (the raw data were not presented so that an independent statistical evaluation could be conducted). No other signs of reaction to diazinon were reported.

This study is SUPPLEMENTARY. The report lacks sufficient detail to upgrade the study.

Study Type: Teratology - Rabbits

Accession No.:

MRID No.: 79013

Sponsor: Ciba-Geigy Co.

Testing Laboratory: Science Applications, La Jolla, Calif.
(Study No. 281005)

Date: July 28, 1981

Review:

The following is quoted from the original review by Dr. W.D. Dykstra dated November 2, 1981.

"Review:

1. A dose range-finding teratology study of Diazinon in New Zealand White Rabbits including a positive control group administered 6-aminonicotinamide (Science Applications, Inc. Study No. 280007; July 28, 1981).

Groups of six pregnant rabbits were administered Diazinon to determine dose levels for the definitive teratology study. The compound was orally administered via gavage at 0 (control) 7, 25, 100, 200, 300, and 400 mg/kg/day from days 6-18 of gestation. All does were weighed on the day of insemination, daily throughout dosing, on day 25 of gestation, and prior to laparohysterectomy on day 30 of gestation. Fetuses were weighed and evaluated for external abnormalities only. A positive control agent, 6-aminonicotinamide, was given orally to 10 pregnant rabbits at 2.5 mg/kg on day 9 of gestation, to demonstrate the sensitivity of this species to a known teratogen.

Results:

Significant maternal mortality was observed at 100 (16.7 percent), 200 (83.3 percent), 300 (33.3 percent), and 400 (50.0 percent) mg/kg. Because the 200, 300, and 400 mg/kg dose levels produced substantial maternal toxicity, these treatment groups were discontinued. Neither embryotoxicity nor frank external malformations were seen as a result of Diazinon exposure. The positive control agent (6-aminonicotinamide) elicited a characteristic embryotoxic response, which included increases in embryoletality, malformations, and decreased fetal weights.

Conclusion:

In conclusion, these results indicate that the maximum tolerated dose that could be used in the definitive teratology study was 100 mg/kg.

Classification: Supplementary Data.

2. A teratology study of Diazinon in New Zealand White Rabbits (Science Applications, Inc., Study No. 281005; 7/28/81).

The compound was orally administered by gavage at 0 (control), 7, 25, and 100 mg/kg from days 6-18 of gestation as shown below:

<u>Group No.</u>	<u>No. Bred</u>	<u>Dose (mg/kg)</u>	<u>Day of Compound Administration</u>
I	19	0.0 (0.2% CMC)	6-18
II	18	7.0	6-18
III	19	25.0	6-18
IV	22	100.0	6-18

All does were weighed on the day of insemination, daily throughout dosing, on day 25 of gestation, and prior to laparohysterectomy on day 30 of gestation. Fetuses were weighed, sexed, and evaluated for external, visceral, and skeletal abnormalities, and variations.

Results:

At the dose of 100 mg/kg, Diazinon produced a biologically significant, but not statistically significant, increase in maternal deaths. Doe mortality was seen only at the 100 mg/kg dose which was lethal to 9 of 22 (40.9 percent) of the females exposed. This effect is statistically significant ($p < .005$) in conventional Chi-Square analysis and approached statistical significance ($p < .07$) when analysis is done by Fisher's Exact Test. Seven of the nine females that died exhibited lesions characteristic of gastrointestinal toxicity. No gross lesions were observed in the remaining two females that died on study. The remaining doses of 7 and 25 mg/kg caused no maternal deaths.

There were no statistically significant differences among the groups regarding the mean number of implantation sites, proportions of live, dead, or resorbed fetuses per litter. Similarly, fetal weights were not significantly different among groups. Furthermore, no alternations in fetal sex ratio were observed.

A small number of miscellaneous malformations were observed in this study that included: Control - one fetus was considered a runt by the definition of a runt being \leq 30 percent of its mean litter weight, one fetus with a common truncus arteriosus and ductus arteriosus arising from the left pulmonary artery, three fetuses with fused sternbrae, one fetus with small clavicles, and one fetus with scoliosis; 7 mg/kg - four fetuses were runts, one fetus had fused sternbrae, and fetus had small clavicles; 25 mg/kg - one fetus was a runt, one fetus had a common truncus arteriosus with two lumens and interventricular septa defect, five fetuses had fused sternbrae, and one fetus had fused ribs; 100 mg/kg - one fetus was a runt. There were no significant differences among control and treated groups and none of the observed malformations were determined to be related to treatment.

A variety of skeletal variations were observed among experimental and control litters but not a statistically significantly greater incidence among groups. The most frequent skeletal variations were the number of fetuses with either 12 or 13 pairs of ribs and incomplete ossification of either the fifth or sixth sternbrae.

Conclusion:

Significant maternal toxicity was observed only in the highest dose group (100 mg/kg). There were no indications of prenatal toxicity/teratogenicity at any of the dose levels studied. Because 100 mg/kg Diazinon approached the maternal multiple-dose LD₅₀ (40.9 percent mortalities) and produced no signs of fetotoxicity, it is inferred that maternal rabbits were more sensitive to the test article than rabbit embryos or fetuses. These findings demonstrated that Diazinon was not fetotoxicity or teratogenic in rabbits at dosages of 100 mg/kg or less.

Classification: Core-Minimum Data."

Study Type: Teratology-Rats

Accession No.: 257826

MRID No.: ~~Not provided~~ 0015 3017

Sponsor: Ciba-Geigy

Testing Laboratory: Ciba-Geigy, Study No. 52-83

Date: April 19, 1985

Review:

The following review was prepared by Dr. W. Dykstra and secondarily reviewed by Edwin Budd. The review is dated March 12, 1986.

Review:

A Teratology Study of Diazinon Technical in Charles River Rats. (Ciba-Geigy report No, 52-83; master index No. 82-2-96; April 19, 1985).

Test Material: Diazinon technical; Batch No. FL-821568.

Randomized groups of 27 pregnant Sprague-Dawley rats received by intubation dosages of 0 (vehicle only), 10, 20, and 100 mg/kg of test material during gestational days 6 through 15.

Dams were observed for toxic signs, body weight and food consumption.

Dams were sacrificed on day 20 of gestation and reproductive parameters were recorded.

Fetuses were weighed, sexed, and evaluated for external abnormalities.

Approximately 1/3 of the fetuses of each litter were evaluated by serial sectioning for visceral anomalies. The remaining two-thirds of the fetuses were cleared, stained with Alizarin Red S and examined for skeletal anomalies.

Statistical evaluations of the data were performed.

Results:

All dams survived the duration of the study and were sacrificed on schedule on day 20 of gestation.

Toxic signs were not compound-related but occurred as alopecia, bleeding from nasal and/or oral mucosa and encrustment about the eyes.

Food consumption was significantly reduced during days 6 to 9 in high dose dams. This finding is considered compound-related.

Body weights of females in the low- and mid-dose group were comparable to controls. The decreased body weight of high-dose females is considered compound-related.

Similarly, body weight gain of high-dose animals was -11 ± 2 grams during days 6 to 10 of gestation in comparison to 14 ± 2 , 14 ± 1 , and 13 ± 1 of control low-, and mid-dose animals, respectively. Body weight gain during days 10 to 14 in high dose females was increased above controls showing recovery. However, the overall body weight gain of high dose females was significantly less in comparison to control animals and is considered treatment-related.

Reproductive parameters showed variation among groups. Comparable incidences among groups of corpora lutea and implantations were observed.

Resorptions were significantly decreased in the mid-dose group and increased in the high-dose group (means: 1.0, 1.0, 0.4, 1.8 of control, low, mid, high).

Additionally the mean number of live fetuses was decreased in the high-dose group (14.2, 13.4, 14.2, 11.9 of control, low, mid, high).

The percent of preimplantation loss was increased in exposed groups (6.9, 10.0, 9.5, 12.5% at control, low, mid, high). Post-implantation loss was 6.9, 7.7, 2.8, and 13.4% for control, low, mid, high. These variations were considered incidental to treatment.

Fetal sex ratio was within normal limits among groups. ;)

Male and female fetal weights were higher in the high-dose group in comparison to controls and the increase was statistically significant. This finding may be considered compound related, but is not considered fetotoxicity.

External evaluation of fetal malformations showed one fetus in the mid-dose group with exencephaly (fetus 6 of dam YN18). Three high-dose fetuses also exhibited major external malformations; one fetus had umbilical hernia (fetus 7 of dam YK15); and one fetus had sublingual extraneous soft tissue.

The appearance of external malformations showed a dose-related trend and a statistically significant incidence at the high-dose.

However, the incidence of external malformations is not considered compound-related since the effects occurred at 100 mg/kg, a dose which produced severe maternal toxicity. Additionally, the three malformations were morphologically unrelated.

Visceral anomalies were observed at the mid- and high-dose. At the mid-dose level, one fetus (fetus 6, dam YN8 had situs inversus.

This finding is considered incidental to treatment.

Visceral findings at the high-dose were 6/81 fetuses with mottled liver in comparison were 0/105 (control), 0/84 (low) and 0/108 (mid). The fetuses with mottled livers appeared in four litters.

Histological evaluation of normal appearing control and high-dose mottled livers showed there were no differences between groups. The occurrence of mottled livers in the high-dose group is not considered a compound-related finding.

Skeletal anomalies appeared as a significant increase in rudimentary 14th ribs. This finding is considered evidence of fetotoxicity rather than terata.

The following table shows the incidences of rudimentary ribs.

<u>Fetuses</u>	<u>Dose (mg/kg)</u>			
	<u>0</u>	<u>10</u>	<u>20</u>	<u>100</u>
No. examined	235	198	247	181
Rudimentary T-14	0	6	6	9*
 <u>Litters</u>				
No. examined	24	21	25	22
Rudimentary T-14	0	3	4	6*

*P < 0.01

The fetal incidence of rudimentary ribs (3% in low, 2% in mid, and 5% in high) are within the range of historical control data. The historical control data ranged from 0.47 to 14.93% during 1982 through 1985.

Other fetal anomalies or fetotoxicity were comparable between control and exposed groups.

Conclusion:

Diazinon was not teratogenic in rats up to 100 mg/kg (HDT). At the high dose, maternal toxicity was evidenced as decreased food consumption, decreased body weight and body weight gain.

Three single instances of external malformation were observed at the high dose (one fetus with filamentous tail, one fetus with umbilical hernia, and one fetus with sublingual extraneous soft tissue). Since the malformations were not morphologically related, and considered secondary to maternal toxicity, these were not considered compound-related.

Fetotoxicity present as mottled liver in 6/81 fetuses in the high-dose are not considered compound-related since, histologically, the livers were within normal limits.

The incidence of rudimentary 14th ribs at the high-dose was within the range of historical control data for this variation. The NOEL for fetotoxicity is 100 mg/kg/day.

Classification: Guideline.

Study Type: Teratology Rat

Accession No.:

MRID No.: 109033

Sponsor: Geigy Corporation

Testing Laboratory: Ciba-Geigy Switzerland
(Study No. PH 2.632)

Date: May 16, 1974

Review:

The following is the original review of this study as prepared by Robert Coberly and dated January 10, 1976.

"The material tested was identified as G24480 (Diazinon Technical). This material was administered to 28 to 30 dams per level of 15, 50, and 100 mg/kg/day through days 6 to 15 of gestation. The fetuses were removed by cesarean section on day 21 of pregnancy.

Observations and tests for effects among the dams included general conditions, body weight gain, food consumption, and assessment of internal organs.

Observations and tests for effects among the fetuses included external examination, body weight, examination of the viscera according to the slicing technique of Wilson, skeletal assessment, and complete organ examination.

Observations and tests for reproductive effects included number of corpora lutea, implantations, abortions, resorptions, fetal deaths, viable fetuses, and fetal weights.

Results:

One fetus of the 50 mg/kg displayed hyponathia inferior and a cleft palate. Some minor skeletal variations were also reported. None of these findings are considered of a teratogenic nature.

The NEL is 100 mg/kg/day (highest level tested)."

This study is being assigned SUPPLEMENTARY status by J. Doherty as of June 1986.

Study Type: Teratology - Rats

Accession No.:

MRID No.: 131150

Sponsor: Trans Chemic Industries, Inc. Secaucus, N.J.

Testing Laboratory: Institute for Animal Reproduction,
Ohmiya, Japan

Date: November 1, 1979

Review: [Note: portions of the available document are unreadable.]

The test material used for this study was diazinon but neither the lot number or purity were stated. The test animals used were Wistar-Imanichi rats (SPF, which were obtained from the testing institute's own stocks). The rats were dosed with either 0, 0.53, 1.45, or 4.00 mg/kg of diazinon or 50 mg/kg of ethylene-thiourea (the positive control). There were thirty pregnant rats per group, twenty were sacrificed on the 21st day of gestation and the remaining ten were allowed to deliver. The test material was emulsified with Tween 80 in water and administered on days 7 to 17 (11 doses). The remainder of this review is divided into two sections: the basic teratology study and the developmental aspect.

A. Basic Teratology Study

1. Maternal Aspects

The study report asserts that there were no effects of diazinon on the dams. [Note: The dose levels selected were based on preliminary studies and were specifically chosen to give a minimum of toxicity to the dams.] There was an unusual finding that food consumption (without concurrent body weight loss) in the high dose test group was decreased.

There were no differences among the dams with regard to number of corpora lutea, number of implants, number of resorptions (early or late deaths), number of live fetuses.

The NOEL is thus > 4.0 mg/kg. It should be noted that in teratology studies, the highest dose group should show some signs of maternal toxicity.

2. Fetal Aspects

There were 270, 255, 261, 263, and 252 fetuses for the control, low, mid, and high dose test groups and for the

positive control group delivered by caesarean section. A single fetus in all of the diazinon test groups was grossly malformed (tailless, anal atresia, and club foot). In contrast 249 out of 252 living fetuses from dams dosed with the positive control were malformed. Except for the low- and high-dose test group males being slightly higher in weight (about 4%), the pups from the diazinon-treated groups were similar to the controls.

None of the skeletal or visceral abnormalities noted in the pups occurred with a sufficient frequency to justify a conclusion that they were related to diazinon treatment.

B. Parturition and Behavioral Studies

Of the 10 rats from each dose group (except for the positive control for teratogenicity group) there were 130, 130, 119, and 124 rat pups delivered. There were no gross or obvious abnormalities in these groups.

Pups from the control and 4.0 mg/kg dose group were selected and subjected to a series of behavioral tests. These tests included light/dark discrimination, spontaneous activity and emotionality.

The pups were allowed to grow until they were 10 weeks of age. The maturity of these rats was monitored and eventually they were mated.

Overall, the conclusions of this aspect of the study were that diazinon had no effects. There were some differences noted in morphological differentiation but these were considered to be technical artifacts related to the time of assessment because the pups developed normally to maturity in spite of some apparent deviations.

Conclusion:

This study is SUPPLEMENTARY. The major deficiency of this study is that the test doses were too low. Thus, the behavioral and maturity aspects of the F₁ generation are of very limited usefulness in assessing for potential behavioral toxicity of pups derived from diazinon dosed dams.

Study Type: 3-Generation Reproduction Rats

MRID No.: 55407

Accession No.: 90392

Sponsor: Geigy

Testing Laboratory: Woodward Research Corporation
(Study No.: None provided)

Date: May 20, 1965

Review:

The following is a copy of the original review of this study as prepared by Dr. George Whitmore and dated June 25, 1965.

"Reproduction Study:

Twenty (20) of each sex as controls and twenty of each sex on 4 ppm Diazinon diets as original parents. Ten (10) males and twenty females were selected from the second litters of the control group and continued as controls. One (1) group of ten males and twenty females was selected from the 4 ppm group and continued on 4 ppm. A second group was selected and placed on a 8 ppm diet and continued at this level.

The observations for effects included number of litters per group, total pups born, litter size, birth weight, survivors to weaning and weaning weight. The F3b weaned rats were necropsied for organ weights, gross and histopathological examination.

	<u>F₀ Generation</u>			
	<u>Controls</u>		<u>Diazinon 4 ppm</u>	
	<u>First Litters</u>	<u>Second Litters</u>	<u>First Litters</u>	<u>Second Litters</u>
Number litters per group	11/20	15/19	18/20	18/20
Total number stillbirths	3	10	3	3
Total number young at birth	117	114	194	216
Number young per litter	10.6	9.6	10.8	12.0
Mean birth weight, grams	5.9	6.3	5.7	6.3
Percent young alive at weaning	68	97	84	95
Mean weaning weight, grams	29.3	33.7	31.0	35.3

F2a Litters

	<u>Diazinon</u>		
	<u>Controls</u>	<u>8 ppm</u>	<u>4 ppm</u>
Number litters per group	18/20	17/20	18/20
Total number stillbirths	3	8	3
Total number young at birth	158	150	160
Number young per litter	8.8	8.8	8.9
Mean birth weight, grams	6.3	6.3	6.4
Percent young alive at weaning	89	93	89
Mean weaning weight, grams	42.1	30.1	39.3

F2B Litters

	<u>Diazinon</u>		
	<u>Controls</u>	<u>8 ppm</u>	<u>4 ppm</u>
Number litters per group	19/19	17/19	11/19
Total number stillbirths	6	18	0
Total number young at birth	189	163	110
Number young per litter	9.9	9.6	10.0
Mean birth weight, grams	6.1	5.8	5.9
[data lost from review]

F3a Litters

	<u>Diazinon</u>		
	<u>Controls</u>	<u>8 ppm</u>	<u>4 ppm</u>
Number litters per group	16/20	16/18	18/20
Total number stillbirths	1	2	10
Total number young at birth	135	154	149
Number young per litter	8.4	9.6	8.3
Mean birth weight, grams	6.3	6.1	6.1
Percent young alive at weaning	88	86	87
Mean weaning weight, grams	34.0	33.2	31.4

F3b Litters

	<u>Diazinon</u>		
	<u>Controls</u>	<u>8 ppm</u>	<u>4 ppm</u>
Number litters per group	18/20	14/16	19/19
Total number stillbirths	5	6	2
Total number young at birth	172	125	203
Number young per litter	9.6	8.9	10.7
Mean birth weight, grams	6.8	6.9	6.6
Percent young alive at weaning	86	90	86
Mean weaning weight, grams	39.1	42.2	35.2

Compound-related organ weight changes and pathology were absent in the necropsied F₃b weaning rats.

It is apparent that a 4 ppm Diazinon diet for breeding rats for three generations was without effect to the reproductive process. It is also apparent an 8 ppm diet was without effect for two generations."

This study is being assigned SUPPLEMENTARY status as of June 1986 by J.D. Doherty.

Reviewed by: John Doherty
Section 2, Tox. Branch (TS-769C)
Secondary reviewer: Edwin Budd
Section 2, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Chronic Feeding - Rat Tox. Chem. No. 342

Accession No.: 90054 MRID No.: 75932

Test Material: Diazinon 25W (Wettable Powder)

Synonyms: O,O-diethyl-O-[2-isopropyl-4-methyl-pyrimidyl
(6)]thiophosphate

Study No(s).: [None]

Sponsor: Ciba-Geigy

Testing Facility: Hazleton Laboratories, Falls Church

Title of Report: "Chronic Feeding"

Author(s): H.J. Horn

Report Issued: December 22, 1955

Conclusions:

Classification: CORE - SUPPLEMENTARY

A. Materials:

1. Test compound: Diazinon 25W, Description Wettable Powder
Batch Nos. E122423, E1#396, Purity 25%, contaminants: not
provided. ;)
2. Test animals: Species: rat, Strain: Carworth Farms,
Weight: 62 to 63 grams.

B. Study Design:

1. Animal assignment - Animals were assigned randomly to the
following test groups:

Test Group	Dose in Diet (ppm)	Main Study		Interim Sac.	
		24 months male	24 months female	--- months male	--- months female
1 Cont.	0	20	20		
2 Low (LDT)	10*	20	20	NONE	
3 Mid (MDT)	100	20	20		
4 High (HDT)	1000**	20	--		

* As diazinon, not total test material.

** Started at 100 and gradually raised to 1000 over a period of 11 weeks.

-- Not tested at this level.

2. Diet preparation - Diet was prepared (information not provided) and stored at unknown temperature. Samples of treated food were analyzed for stability and concentration at 9 intervals.

Results - The analysis of the test material was done by the sponsor who reported the samples were 22.7 to 24.4 percent active (diazinon). No other results of analysis of the diet as reported except during the 55th week the diazinon was found to be only 3.3 percent active meaning the rats received diazinon at levels below their test diet concentrations at least during some periods of this study.

3. Animals received food (unspecified) and water ad libitum.
4. Statistics - The following procedures were utilized in analyzing the numerical data: usually "t" test.
5. Quality assurance: None [study is ca. 1953 to 1955].

C. Methods and Results:

1. Observations - Animals were inspected at unspecified intervals for signs of toxicity and mortality.

Results - Toxicity

Mortality (survival) - No adverse effects on survival were noted for either sex. There were 50 percent or more survivors in each group.

2. Body weight -

Results - Terminal body weight were unaffected for all male groups. The high-dose female group (100 ppm) was about 9 percent less than the corresponding control group.

3. Food consumption and compound intake -

Results - Food consumption -

These parameters were not affected by the test material.

4. Ophthalmological examinations

- None performed.

5. Blood was collected and the CHECKED (X) parameters were examined.

a. Hematology -

$\frac{X}{\bar{X}}$	Hematocrit (HCT)*	$\frac{X}{\bar{X}}$	Total plasma protein (TP)
	Hemoglobin (HGB)*		Leukocyte differential count
	Leukocyte count (WBC)*		Mean corpuscular HGB (MCH)
	Erythrocyte count (RBC)*		Mean corpuscular HGB conc. (MCHC)
	Platelet count*		Mean corpuscular volume (MCV)

Results - It is possible that the data show effects of diazinon on the hematocrit levels at all dose levels tested for both sexes. The study report, however, recognizes that only the high-dose group males were statistically significant. The following table illustrates the results.

	Males	Females
Control	53.7	51.9
Low	44.2 (-18%)	47.7 (-8%)
Mid	49.0 (-9%)	46.7 (-10%)*
High	44.8 (-17%)	--

* Reported to be statistically significant.

b. Clinical Chemistry - At termination only.

<u>X</u>	Electrolytes:	<u>X</u>	Other:
	Calcium		Albumin
	Chloride		Blood creatinine
	Magnesium		Blood urea nitrogen
	Phosphorous		Cholesterol
	Potassium		Globulins
	Sodium		Glucose
	Enzymes		Total bilirubin
	Alkaline phosphatase		Total protein
X	Cholinesterase		Triglycerides
	Creatinine phosphokinase		
	Lactic acid dehydrogenase		
	Serum alanine aminotransferase (also SGPT)		
	Serum aspartate aminotransferase (also SGOT)		

Results - The following table illustrates that plasma and RBC cholinesterase was inhibited at all dose levels of diazinon.

Table No. 129* - Percent of normal cholinesterase activity found in the plasma, red blood cells, and brain of male and female albino rats receiving the basic laboratory diet or the basic diet containing 10, 100, or 1000 ppm (0.001%, 0.01%, or 0.1%, respectively) Diazinon Wettable Powder for a period of two years.

Level ppm	Sex	No. of Rats	Percent Activity		
			Plasma	RBC	Brain
Control	M	5	--	--	--
	F	5	--	--	---
10	M	5	40	76	90
	F	5	33	58	92
100	M	5	5	2	81
	F	5	2	0	47
1000	M	5	0	0	41

* Reproduced from the study report.

The values for plasma and RBC are significant at all dose levels, but the brain decreases did not reach statistical significance until the mid dose level.

6. Urinalysis - No urinalyses were performed.

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7. Sacrifice and Pathology - Only 5 males and 5 females from each group were sacrificed at termination and prepared for histological examination. The X organs were examined, the XX organs were weighed.

<u>X</u>	Digestive system	<u>X</u>	Cardiovasc/Hemat.	<u>X</u>	Neurologic
	Tongue		Aorta		Brain
	Salivary glands	X	Heart		Periph. nerve
	Esophagus	X	Bone marrow		Spinal cord
X	Stomach		Lymph nodes		(3 level)
XX	Liver	X	Spleen		Pituitary
	Gall bladder		Thymus		Eyes (optic n.)
X	Pancreas		Urogenital		Glandular
X	Large and small intestine	XX	Kidneys	X	Adrenals
	Respiratory	X	Urinary bladder		Lacrimal gland
	Trachea	X	Testes		Mammary gland
	Lung		Epididymides		Parathyroids
			Prostate	X	Thyroids
		X	Seminal vesicle		Other
			Ovaries		Bone
			Uterus		Skeletal muscle
					Skin
					All gross lesions and masses

Note: Only the organs from 3 animals were actually examined.

Results:

- a. Organ weight - Only the kidney and the liver were, actually weighed. The study report asserts that no effects of diazinon were evident although liver weight for the 1000 ppm dose group males was lower (-3 to 4%).
- b. Gross pathology - No test chemical effects noted.
- c. Microscopic pathology - The conclusion of the report was that diazinon had no effect on the cellular structure. Because too few animals per dose group were assessed, this study does not qualify as an oncogenicity evaluation.

D. Discussion:

The study is CORE SUPPLEMENTARY as a chronic feeding study. The study is deficient in both assessing hematology, clinical chemistry, urinalysis, organ weight variation, and both macro and histopathology. This study is typical of the procedures used in 1955 but does not meet current standards of testing. The test data indicate possible effects on the hematocrit and definite effects (NOEL < 10 ppm) in the plasma and RBC ChE activity. At 100 ppm the brain ChE levels are also decreased. There were also apparently problems with maintaining the stability of the test material such that diazinon decomposed during the testing period.

There were insufficient animals prepared and assessed to make any conclusions regarding the potential for diazinon to cause cellular structural changes or neoplasia.

Study Type: Oncogenicity - Rats (National Cancer Institute Sponsored Study)

Accession No.:

MRID No.: 73372

Sponsor: National Cancer Institute

Testing Laboratory: Gulf South Breeze Research Institute
(Study No. 79-1392).

Date: 1979

The test material was diazinon (see the review of the mouse oncogenicity study by NCI in this Registration Standard on page R-36 for a more complete description of the test material used).

The test animals used were Fischer F344 rats obtained from the NCI Frederick Cancer Research Center Frederick, MD. The rats were approximately 7 weeks of age at the start of the study. There were 25 males and 25 females in the matched control groups and 50 males and 50 females in each of two dosed groups which received either 400 or 800 ppm of diazinon in the diet. The dosing period was for 103 weeks which was followed by a 2-week observation period. Note: The dose levels used were selected following a subchronic range-finding study which showed toxicity (body weight loss) at 1600 ppm (in females) and some deaths at 3200 ppm.

Survival: There was no increase in deaths due to diazinon in the diet in either sex. In fact, there were more survivors in the male high-dose group. There were greater than 44 dosed rats per sex and 23 control rats per sex which survived 78 weeks of dosing, thus being at risk for late tumor development.

The clinical signs noted included that "tissue masses were observed at highest incidences in high dose males and low dose females, and tachypnea was observed at a higher incidences in dosed groups than in control groups." Other symptoms in the dosed rats were "hyperactivity, discolored urine (high-dose group), "bloating," "vaginal bleeding," and "vaginal discharge." None of these were quantitated or presented as frequency of occurrence or relative intensity. It is not possible to set NOEL's and LEL's from the available information.

There were no hematology, clinical chemistry, or urinalysis determinations made. There were no organ weight determinations made.

There was no summary table showing the gross necropsy findings and since individual animal pathology sheets were not presented, it could not be determined if gross necropsy observations were followed up microscopically.

The report conclusion for this study is "histopathologic examination provided no convincing evidence for carcinogenicity of diazinon in F344 rats under conditions of this bioassay." The data as presented appear to support this conclusion.

Conclusion:

This study is SUPPLEMENTARY. A second study will have to be provided by the sponsor to meet the requirement for oncogenicity testing in rats. The study as presented is lacking in sufficient detail to justify a CORE MINIMUM or higher rating.

Study Type: Oncogenicity - Mouse (National Cancer Institute Sponsored Study)

Accession No.:

MRID No.: 73372

Sponsor: National Cancer Institute

Testing Laboratory: Gulf South Breeze Research Institute

Date: 1979

Review:

The test material used for this study was diazinon and was obtained from the Ciba-Geigy Co. The identity and purity of the batch (Lot No. F1-741308) was said to be confirmed by the Gulf-South Research Institute and the material was said to be 98 percent pure. The report stated that independent analysis of the material did not indicate changes in composition on storage for 4 years. The conditions of storage and results of analysis were not provided in the report.

The test animals used in this study were B6C3F1 mice which were obtained from the NCI Frederick Cancer Research Center. The mice were approximately 6 weeks of age when placed on the study and were dosed for 103 weeks. There were 25 of each sex for the control groups (concurrent) and 50 of each sex for the diazinon treated groups which were dosed with 100 (low dose) and 200 (high dose) ppm. The dose levels were selected based on the results of subchronic feeding studies which showed weight loss at 800 ppm and deaths for all mice at 1600 ppm and above. Diazinon was more toxic to the mice than to the rats. The mice were observed for 2 weeks after stopping the test diet, thus, the survivors were 105 weeks of age at sacrifice.

Survival: There was no trend toward dose related increases in death in either sex (Tarone test, report analysis). There were 98 percent, 90 percent, and 84 percent survivors among the males and 98 percent, 100 percent, and 96 percent survivors among the females for the high-, low-, and control groups which survived to week 76. Thus, there were nearly 45 to 50 mice of each sex per dosed group and 21 to 24 controls at risk for late developing tumors.

The body weight gains for all dosed male mice were reported as being similar to the control group. The female mice were reported to be slightly lower in weight than the controls for the last 20 weeks of the bioassay. The report also states that "hyperactivity" was noted in the dosed mice but was rare in the control groups. There was no table or summary of the incidences of hyperactivity presented.

No hematology, clinical chemistry or urinalyses were determined. No organ weights were made.

Pathological Findings: No table summarizing the gross necropsy findings was available and since there were no individual animal pathology sheets attached, TB could not verify if gross necropsy lesions were followed up by microscopic analysis. The microscopic pathology summary tables indicate that most of the tissues/organs were examined for the mice.

The study report concluded that "histopathologic examination provided no convincing evidence for the carcinogenicity of diazinon in B6C3F1 mice under the conditions of this bioassay."

The data provided support this conclusion. Some individual organs are discussed below:

1. Liver. In the males the low-dose group had 20 incidences in 46 mice examined of hepatocellular carcinomas and the control group had only four incidences among 21 mice examined. When compared to the control group the low-dose group was statistically significant. The high-dose group had 10 incidences of 48 mice examined and was not statistically different. The higher frequency in the low-dose group is considered by TB to be incidental. Moreover, the combined incidences of hepatocellular carcinomas and adenomas was not statistically significant at either the low- or mid-dose levels.

TB also notes that the historical control data indicate that male B6C3F1 mice have this tumor over the range of 16 to 58 percent; the 43 percent incidence noted in this study is within historical control limits.

The other tumor types noted were commonly occurring neoplasms. There were no dose-related increases in lymphomas or leukemias.

Conclusion:

This study is CORE MINIMUM. The study report which is a summary of the original data (no original data were examined by TB) presents evidence that the mice were dosed for 103 weeks and that no indications of diazinon related neoplasia resulted. Since the study was sponsored by the National Cancer Institute, a neutral organization, TB is not requesting the original data of the study. It should be recognized that the maximum tolerated dose was not reached for the males and may have been reached only for the females. The data provide a basis for the conclusion that diazinon was not associated with increased incidences of neoplasms at dose levels up to and including 200 ppm.

Study Type: Mouse Oncogenicity

Accession No.:

MRID No.: 50291

Sponsor: Ciba-Geigy

Testing Laboratory: Industrial Biotest (Study No. 8580-09381)

Date: July 7, 1980

Review:

This study was audited by EPA's contractor (Dynamac Corporation) and determined to be INVALID. (Refer to copy of the IBT Validation Report attached.)

IBT VALIDATION REPORT

- (1) CHEMICAL: Diazinon.
- (2) TYPE OF FORMULATION: Technical.
- (3) CITATION: IBT No. 8580-09381. Carcinogenicity Evaluation with Diazinon Technical in Albino Mice. July 7, 1980.
- (4) SPONSOR: CIBA-GEIGY Corporation.
- (5) EPA ACCESSION NUMBER and/or Pesticide Petition No. and/or Registration No. for this IBT Report: None provided.

(6) VALIDATION PERFORMED BY:

John R. Strange, Ph.D.
Department Director
Dynamac Corporation

Signature: John R. Strange

Date: 23 July 1982

Cipriano Cueto, Ph.D.
Program Manager
Dynamac Corporation

Signature: Cipriano Cueto

Date: 23 July 1982

- (7) Based upon findings listed in this Dynamac Corporation validation report (which included examination of the microfiched raw data, the sponsor's validation report, the final test report, and the SPRD preliminary report when available), I concur with this validity determination.

Signature: Laurence D. Philbeck

Date: 7/26/82

- (8) TOPIC: This study has information pertinent to the discipline of toxicology; topic, oncogenicity. It relates to the Proposed Guidelines data requirement 163.83-2.

(9) VALIDATION REPORT CONCLUSION:

 VALID

 SUPPLEMENTARY

 X INVALID

SUMMARY

A SPRD preliminary report was not present for this study.

The sponsor's validation report noted that the interval between diet preparations was often long and that the test material was unstable in the test diet (Deficiency 1). The report also noted that acetone was used to prepare the control diets instead of corn oil, which was used to prepare the test diets (Deficiency 4). The sponsor validator also reported that checks for tumor incidence were not done during the first 11 months of the study (Deficiency 6), that some of the animals were dusted with Rotenone (Deficiency 7), and that the animals were moved twice during the study (Deficiency 5). However, the sponsor validator found this study "sufficient to evaluate the carcinogenic potential of Diazinon technical."

This review determines the study to be invalid because of the lack of verification that the test animals received the required dosing during the first 8 months of the study, and the high mortality and autolysis among control and test animals which resulted in an extensive reduction in the number of animals and tissues available for pathologic examination. In addition the acetone vehicle control group was not an appropriate control for this study and the animals were moved twice from one laboratory to another during the study.

DEFICIENCIES AND DISCREPANCIES NOTED DURING COMPARISONS
OF THE FINAL REPORT, PROTOCOL, AND RAW DATA

1. Administration of the test material at the dietary levels indicated by the final report procedures could not be verified, for the first 8 months of the study because of the following 4 deficiencies.
 - a. Although the protocol specified that the test diet should be mixed every 7-10 days, there was a lack of documentation of diet formulation during the first 8 months of the study. A diet preparation listing recorded six dates of diet preparation during this period (arrows on Reference 1). Two of these recordings were entries for the same date (6/23/77). According to a letter from IBT to CIBA-GEIGY dated 6/28/77, there were four actual dates of diet preparation in the first 32 weeks of the study (arrow on Reference 2 page 1). The same letter also reported three estimated dates of diet preparation (see dates with asterisks indicated by arrow on Reference 2 page 1). However, there was no documentation of any of these dates of diet preparation contained in the diet formulation records. The first record of diet formulation contained in the raw data is dated 7/6/77, 33 weeks after initiation of the study (arrow on Reference 3). Although the raw data calculations indicated that sufficient diet was mixed each time to last approximately 4-5 weeks, there remained at least a 10-week period for which compound preparation could not be verified during the first 8 months of the study.
 - b. The test material appeared to be unstable in the diet when stored at room temperature for a 7-14 day period. A letter from CIBA-GEIGY to IBT, dated June 2, 1977 (approximately 6 months after the initiation of the study) stated that the test material in the diets may deteriorate over a period of 7-14 days at room temperature and requested that the diets be frozen between mixings (arrow A on Reference 4). The only confirmation that the diet was stored frozen was a comment to that effect on a page of interoffice correspondence dated 6/29/77 (arrow on Reference 5). Additional diet stability information presented in the sponsor validator's report suggested that a substantial degradation of Diazinon in the test diet probably occurred in the intervals between diet preparation during the first 8 months of the study (arrow on Reference 6).
 - c. A limited number of dietary analyses were performed during the first 8 months of the study. Diet analysis data for this time period revealed that concentrations of Diazinon in the T-I diets at 0 and 3 months were approximately 25% below the required level (arrow A on Reference 7). However, because diet analysis data were available for only 0-, 3-, and 6-month samples, the actual dietary levels of test material administered during most of the first 8 months could not be determined.

- d. No records of compound administration were present between 11/17/76 (the start of the study) and 10/27/77 (week 49). Records of feed changes were present on daily observation pages beginning on 10/27/77 (week 49) to 4/6/78 (week 72) (e.g., arrow on Reference 8) and on weekly feed change forms from 4/6/78 to the end of the study (e.g., arrow on Reference 9). Since records of compound administration were not present for the first 11 months of the study, it could not be verified that test diets were administered during this period.

Because of the infrequent diet preparation, poor stability of the test material in the feed, availability of only limited diet analysis data, and the lack of records of compound administration, it could not be verified that the animals received the required concentrations of test material in the diet during the first 8 months of the study.

2. Poor animal survival was reported among all control and experimental groups and resulted in early termination of the study. Although the protocol called for a 24-month study to be conducted (arrow A on Reference 10, page 1), male animals were terminated after 18 months and female animals after 19 months (arrow on Reference 11) due to approximately 80 percent mortality. Attachment A shows graphs of the number of survivors as months on study for male and female control and T-III groups. The graphs indicate a high rate of mortality for male animals between the 15th and 17th months. By 17 months less than 50% of the animals in the male control and T-III groups remained. By 18 months, only 22 (37%) and 16 (27%) of the initial 60 animals in each of the male control and T-III groups remained and the male animals were terminated. Mortality was also high among the female control and T-III groups, but occurred during a slightly later time period.

The graphs in Attachment A indicate that mortality was high among the female control and T-III during the 18th and 19th months. In the female control group 39 (65%) and 25 (42%) of the initial 60 animals remained after 18 and 19 months, respectively. In the female T-III group, 34 (57%) and 18 (30%) of the initial 60 animals remained after 18 and 19 months, respectively. The female animals were terminated during the 19th month due to poor animal survival.

3. Although the protocol indicated that microscopic examinations were to be conducted upon 39 tissues of all surviving mice in the control and T-III groups as well as all mice from these groups which died during the study (arrow on Reference 10, page 2), the number of tissues actually examined histopathologically was considerably less than the number specified by the protocol.

- a. The histopathologic report by EPL acknowledged that varying degrees of autolysis were present in animals that died during the study, but concluded that "limited histopathologic examinations of most of these tissues could be performed." However, the gross pathology performed at IBT indicated that the degree of autolysis was rather severe. For example, approximately 45% of the females in the control and T-III groups (see Attachment E, autolysis column) were indicated as having an autolysis grading of 3 or higher (on a scale of 1 to 4) on the gross pathology sheets (e.g., arrow A on Reference 12). A tissue inventory, for the control and T-III group females (Attachment B, pages 1-4), indicated that autolysis was most frequently reported for the eye and the gastrointestinal tract. However, significant amounts of autolysis were reported for the urinary bladder and mesenteric lymph node in the female control group (13% and 15%, respectively), and for the pancreas, liver, spinal cord, and urinary bladder in the female T-III group (12, 10, 10, and 10%, respectively).
- b. There were a considerable number of tissues, not designated as autolyzed, for which no histopathologic data were present. Major tissues in the female control group for which considerable data were missing included: mammary gland (42%), mesenteric lymph node (37%), and urinary bladder (15%). Other tissues with large percentages of data missing in the female control group included: gallbladder, sternum (bone), bone marrow (femur), mesentery, and thymus. In the female T-III group the following major tissues had large percentages of data missing: mesenteric lymph node, mammary gland, urinary bladder, ovary, brain (cerebellum), and brain (pons). Other tissues in the female T-III group with large amounts of missing data included: sternum (bone), gallbladder, mesentery, and thymus.

Major tissues for which substantial percentages of the total number of tissues were not examined, due to autolysis or a lack of histopathological examination, are shown on Attachment B, pages 1-4 and included mesenteric lymph node, mammary gland, and urinary bladder for the female control group (52, 42, and 28%, respectively). Other tissues in the female control group with large percentages of tissues not examined included: sternum (bone), gallbladder, bone marrow (femur), thymus, mesentery, ileum, caecum, duodenum, jejunum and the eye (100, 100, 100, 98, 98, 53, 43, 38, 35, and 27%, respectively). In the female T-III group the following major tissues had substantial percentages of the total number of tissues not examined: mesenteric lymph node, mammary gland, urinary bladder, brain (cerebellum), brain (pons), and liver (47, 37, 28, 18, 18, and 17%, respectively). Other tissues in the female T-III group with large percentages of the total number of tissues not examined included: sternum (bone), gallbladder, thymus, mesentery, bone marrow (femur), ileum, caecum, duodenum, jejunum, eye, and parathyroid with 100, 98, 97, 97, 90, 55, 47, 43, 38, and 35%, respectively. Therefore, the extent of tissues not examined was considerable and may have prevented the detection of certain tumors.

4. Although the final report stated that the control group was a vehicle control (arrow A on Reference 13), the vehicle used to prepare the control group diets was different from that used to prepare the test diets. The control group was a common control with Supracide and the control animals and the animals in the Supracide carcinogenicity study received diets containing acetone, while the Diazinon test animals received diets containing corn oil as the vehicle for the test compound (arrow A on Reference 14). Thus the common control was not an appropriate control group for the Diazinon study.
5. Although not addressed in the sponsor validation report, all the animals in each group were moved twice during the study. As indicated in an IBT draft version of the final report, both moves involved a distance of over 5 miles (arrow on Reference 15). This draft report indicated that the animals were initially moved after 4 months on study; however, no documentation of this move was present in the raw data. The second move was made after 17 months on study and was well documented (arrow on Reference 16). Mortality and clinical observation records indicated that the moves did not cause any apparent increased mortality or ill health.
6. Although the protocol required that all animals be examined weekly for the presence of tissue masses (arrow B on Reference 10, page 1), these weekly examinations were not initiated until the 11th month of the study (arrow B on Reference 13).
7. Although not mentioned in the final report procedures, some of the animals were dusted with 1% Rotenone to combat a mite infestation (arrow on Reference 17). Clinical observations and mortality records did not suggest that the animals were adversely affected by the treatment.

Study Type: Chronic Dosing - Dogs

Accession No.: 90054

MRID No.: Not Provided

Sponsor: Geigy (Ciba-Geigy)

Testing Laboratory: Hazleton (No study number)

Date: October 20, 1954

Review:

This study is unacceptable for regulatory purposes. Only one mongrel dog per sex per dose level was used. The test material used was a 25 percent wetttable powder of unidentified composition. The dose level was in units of diazinon. ChE inhibition was noted at all dose levels (LEL 4.6 mg/kg/day). The study otherwise reveals no other meaningful data except that this high-dose group exhibited decreased appetite, weight loss and "depression" and the test material had to be withdrawn.

This study is SUPPLEMENTARY.

Study Type: Chronic Administration (Gavage) - Monkeys

Accession No.: 90737

MRID No.: 57664, 64320, and 64319

Sponsor: Ciba-Geigy

Testing Laboratory: Woodward Research Corporation

Date: June 1, 1966 (As submitted by study authors)

Review:

- B. The test material used for this study was Diazinon 50W; a wettable powder formulation of undescribed composition. It was from Lot No. 72237 and was said to be 48.6 percent diazinon. No records of analytical assessment of the test substance were provided with the report.
- C. The test animals used were rhesus monkeys (Macaca mulatta). They were obtained from three different suppliers and no information on their age at the start of dosing was provided (although their initial body weights were 2.04 to 2.56 kg). Twenty-four monkeys (12 males and 12 females) were used for this study and were grouped as 3 per sex per dosing group. The test material was administered by stomach tube 6 days per week for the scheduled 104 weeks (2 years). The dose levels administered were 0, 0.05, 0.5, and 5.0 mg/kg/day. Initially the monkeys were started at dose levels of 0.1, 1.0, and 10.0 mg/kg/day but due to toxicity of diazinon the levels were reduced over the period of 19 to 34 days.
- D. Survival: All but four monkeys survived. One of the monkeys died of apparent suffocation in its own body fluids. The other three died of apparent intercurrent infection. The study report maintains that there were no compound-related deaths. No evidence of compound-related death was found by TB reviewer.
- E. The monkeys were observed daily for clinical signs and behavioral reactions. There were some signs of "soft stools" primarily seen in the mid- and high-dose test groups. Another sign which was not definitely related to the test material was "hyperesthesia" (abnormally increased sensitivity).

On this basis a NOEL of 0.05 mg/kg is suggested.

- F. Body weight: The report states that the control group showed a slightly greater weight gain than did the treated animals. There were too few monkeys per dose group to determine if there was other than a pronounced effect on body weight gain, food, or water consumption.

[Note: For sections G (Hematology), H (Clinical Chemistry) assessments were made at weeks 0, 3 to 4, 6, 9, 14, 20, 26, 39, 53, 66, 78, 91, and 104. Urinalyses were made at weeks 65, 78, 93, and 104.]

- G. Hematology consisted of assessing Hb, hematocrit, sedimentation rate, WBC count and differential WBC count.

No consistent compound-related changes in any of the hematology parameters were evident.

- H. Clinical Chemistry consisted of assessing serum alkaline phosphatase, glucose, glutamic pyruvic transaminase, BUN, and glutamic oxalacetic transaminase.

No consistent compound-related changes in any of these clinical chemistry parameters were evident.

Cholinesterase Determinations:

The following table depicts the red blood cell and plasma ChE data for the last four determinations that were made. (This table was reproduced from the study report.)

Week	Dosage Level							
	Control		5.0 mg/kg		0.5 mg/kg		0.05 mg/kg	
	A	I	A	I	A	I	A	I
	<u>Median Plasma Cholinesterase</u>							
66	1.32	0	0.25	82	0.22	84	1.23	8
78	1.26	1	0.22	84	0.72	46	1.21	9
91	1.36	0	0.19	86	0.26	82	1.20	10
104	1.30	0	1.06	20	1.19	8	1.27	1
	<u>Median Red Blood Cell Cholinesterase</u>							
66	1.20	0	0.15	88	0.80	33	1.20	0
78	1.22	0	0.35	70	1.06	7	1.21	0
91	1.36	0	0.38	67	1.29	0	1.34	0
104	1.24	0	0.44	62	1.14	1	1.18	0

A = Activity in Δ pH per hour.

I = Percent inhibition (based on 0 time)

In addition to the blood ChE, brain ChE determinations were also made. Only a single high-dose group female had decreased brain ChE activity (67% lower than the control) that was recognized by the test report. Two other monkeys which died during the course of the study also had depressed brain ChE (both were males and were -68% and -70%) but this

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depression could not be conclusively related to the deaths of the monkeys or to diazinon.

Overall, the NOEL for ChE inhibition is set at 0.05 mg/kg. This conclusion is supported by the interim reports for 6 months and 1 year. The inhibition of plasma ChE that occurs at 0.05 mg/kg is not considered to be of such a sufficient degree or consistency to justify setting the NOEL as < 0.05 mg/kg/day.

- I. Urinalysis. The parameters investigated consisted of "appearance," specific gravity, pH, albumin, sugar and microscopic observation.

No consistent compound-related changes in any of these parameters were noted.

- J. Organ Weights. The liver, heart, thyroids, brain, kidneys, lung, pituitary, adrenal, gonads, prostate or uterus, and seminal vesicles were weighed following sacrifice.

No compound-related changes were evident, but due to the small sample size (2 to 3 sex per group) only major changes in organ weight would have been noted.

- K. Gross Pathology. The principal finding of the gross necropsy was that lesions were formed which allowed the deaths of three of the monkeys to be attributed to infections. There were no chemical related lesions reported.

- L. Histopathology. Dr. M.T.I. Cronin of New Haven, CT, evaluated the tissues from this study. His conclusion was that he was "unable, on the basis of these histopathological observations, to detect in the sacrificed treated monkeys any effect of compound administration at any of the three dosage levels employed."

TB notes that since there were only three monkeys per sex, per dose group and that more organs/tissue types were assessed in the control and high-dose test groups than in the low- and mid-dose groups only pronounced effects would be detectable.

- M. Ophthalmoscopic examination (at termination) included observation of the intraocular tension as estimated by palpation, pupillary reflex, and condition of the conjunctivae, eyelids, cornea, aqueous humor, iris, lens, vitreous humor, fundus and sclera.

No abnormal or chemical related effects were noted.

Neurological examination revealed normal motor and sensory activity, reflexes, and reactions for all treated and control monkeys according to the study report.

Note: No data tables were presented for either the ophthalmoscopic or neurological examination results.

- N. Conclusions: This study is SUPPLEMENTARY. It provides useful information on the susceptibility of monkeys to diazinon and identifies ChE inhibition as the sensitive indicator for toxicity to this substance.

The study is limited because only three monkeys of each sex per dose group were used and some of these died before study termination. Thus only pronounced effects of diazinon would have been detected.

One-liner conclusion:

NOEL = 0.05 mg/kg/day
LEL = 0.50 mg/kg/day - plasma and RBC ChE inhibition, possible gastrointestinal disturbance (soft stools) related to ChE inhibition.

Study Type: Sensitization (Humans)

Accession No.: 232008

MRID No.:

Sponsor: Ciba-Geigy

Testing Laboratory: Hill Top Research Institute

Date: December 17, 1963

Review:

The test materials used for this study was 1% Diazinon emulsified into water with Tween 80 or a preparation of Diazinon 2E emulsion (2%) was further diluted with 98 ml of water. The test preparations of diazinon were prepared 2 hours before application.

The test consisted of application of the test materials as 0.5 ml applied to a Warbil swatch and kept in place (upper arms of the volunteers) for 24 hours. A series of nine induction applications were made at 2- to 3-day intervals and the challenge application was made about 14 days after the last induction application. The induction applications were made at the same sites (unless a local irritation developed). The challenge applications were made at the same site and at an adjacent site which did not previously receive the test material.

The volunteers used for this study (27 males and 29 females, total 56 persons) were members of a nearby church. Five volunteers started the program but dropped out due to problems unrelated to reactions to diazinon (as stated in the report). The ages of the volunteers ranged from 16 to 80 with most people being 30 to 49 years old.

Results:

Six of the fifty-six subjects who completed the study "exhibited reactions indicative of sensitization following application of the final challenge patch of both Diazinon 4E and technical diazinon." These subjects were retested and at retest also gave a positive sensitization response in five of the original six subjects which showed a sensitization response in the first phase of the study. A subsequent third test also resulted in a positive reaction to diazinon.

The study conclusion was that "Diazinon included a fairly persistent sensitization."

Discussion:

This study is ACCEPTABLE^{and} provides a basis for classifying diazinon as a potential dermal sensitization agent at least in some persons.

It should be noted that the protocol for this study states that chlorobenzilate and Arugol were studied concurrently but no data were provided and it is unclear if the same persons were tested for all three chemicals.

Study Type: Mutagenicity - Several Assay Types (bacterial gene mutation, repair in microbial and mammalian test systems)

Accession No.:

MRID No.: 132952

Sponsor: U.S. EPA

Testing Laboratory: SRI International (Contract No. 68-01-2458)

Date: October 1979

Review:

In this study 18 pesticides were assessed for mutagenic or genotoxic effects in the following study types.

- o Reverse mutation in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 and in Escherichia coli WP2 uvrA.
- o Induction of mitotic recombination in the yeast Saccharomyces cerevisiae D3.
- o Relative toxicity assays in DNA repair-proficient and deficient strains of E. coli (strains W3110 and p3478, respectively) and of Bacillus subtilis (strains H17 and M45, respectively).
- o Unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells).

The study report asserts that diazinon "was not genotoxic or mutagenic in any of the six assays we performed."

The following is an overview of the acceptability or unacceptability of each of the six study types.

1. Reverse mutation in Salmonella typhimurium strains TA1535, 1537, 1538, 98, and 100 without and with metabolic activation (rat liver S-9 mix from Aroclor 1254 treated rats). The cultures were obtained from the laboratory of B.N. Ames and appropriate positive controls (specific mutagens) were used for each portion of the assay. Diazinon was tested in two separate and independent experiments. The second experiment assessed diazinon at 0, 10, 50, 100, 500, 1000, and 5000 ug/plate and the first experiment assessed diazinon at 0, 1, 10, 50, 100, 500, and 1000 ug/plate. No evidence that diazinon was mutagenic in either of the tests was presented. The specific mutagens (positive controls) produced the expected positive results.

This study is Acceptable. Although no information was presented on the cellular toxicity of diazinon, the highest dose level tested was 5000 ug/plate and this level apparently did not show toxicity.

2. Reverse mutation in Escherichia coli WP2 uvrA. The procedure was provided by reference and was described as being similar to the procedure used with S. typhimurium above (the substitutions and exceptions were indicated). Diazinon was tested in two experiments. The first at dose levels of 1, 10, 50, 100, 500, and 1000, the second at dose levels of 10, 50, 100, 500, 1000, and 5000 ug/plate. Both experiments were run with and without metabolic activation (rat S-9 mix) and appropriate positive controls were included. No indications of a positive response was noted for diazinon. The positive controls responded as expected.

This study is ACCEPTABLE.

3. Induction of mitotic recombination in the yeast Saccharomyces cerevisiae D3. The procedure used was briefly described and the dose levels of diazinon tested were 0.1, 0.5, 1.0, and 5.0 percent concentration (in experiment 1); 1.0, 2.0, 4.0, and 5.0 percent concentration (in experiment 2); and 1.0, 3.0, 4.0, 5.0 percent concentration (in experiment 3). The experiments were run with and without metabolic activation (rat liver S-9 mix) and appropriate positive controls were included. In the first experiment there was an apparent effect of diazinon resulting in slightly higher numbers of recombinants up to 1.6 fold without activation and 1.8 fold with activation, but this effect was not evident in two repeated trials. The false apparent positive result in one experiment was much less than the positive control (1,2,3,4-diepoxybutane) results which were 340 to 464 fold higher than the negative control.

This study is ACCEPTABLE.

4. Relative toxicity assays in DNA repair-proficient and deficient strains of E. coli (strains W3110 and p3478). The assay procedure was described briefly and diazinon was tested at dose levels of 0.01, 0.10, 1.0 and 5.0 mg/disk. At each dose level the zone of toxicity was equal for both strains (repair deficient and repair proficient) of E. coli. The positive control (1-phenyl-3,3-dimethyltriazine) showed a differential of 1.54 to 1.8.

This study is ACCEPTABLE.

5. Relative toxicity in DNA repair proficient and deficient strains of B. subtilis (H17/M45). The assay was similar to the above and the same concentrations of diazinon were tested. Again there was no zone differential for diazinon treated disks; the positive control (1-phenyl-3,3-dimethyltriazine) responded as expected.

This study is ACCEPTABLE.

6. Unscheduled DNA synthesis (UDS) in human fibroblasts (WI-38 cells). The theory and techniques used were described. Diazinon was tested at dose levels of 0, 0.1, 1.0, 10, 100, and 1000 ug/ml with and without metabolic activation (S-9 mix prepared from mouse liver). [Note: Some compound precipitation was noted at 100 and 1000 ug/ml.] In the presence or absence of metabolic activation diazinon resulted in readings (dpm/ug DNA) equivalent to or less than the solvent control (less than apparently indicates toxicity). The positive controls (4-nitroquinoline-N-oxide in the absence of activation and dimethylnitrosamine in the presence of activation) resulted in the expected positive responses.

This study is ACCEPTABLE.

Notes on TB position on accepting these studies. Although in some cases the exact details of the assays are not thoroughly presented, the studies appear to have been well conducted and the positive and negative controls gave their expected results. Some of the other 18 pesticides tested resulted in positive responses in some of these tests. Only the results with diazinon will be discussed in this review. In the studies run with diazinon, a wide range of concentrations were tested sufficient for TB to conclude that high enough concentrations ("limit doses") were used. Most of the studies consisted of two separate independent, repeat experiments which gave similar results. In the one study in which the first experiment gave a suspected positive finding for diazinon, two additional experiments were run (providing negative results) to assure that the first was a false positive.

Study Type: Mutagenicity - E. coli Diffusion Test

Accession No.:

MRID No.:

Sponsor:

Testing Laboratory: Tierfarm AG, Switzerland

Date: November 9, 1970

Review:

Technical diazinon was tested by the paper disk method (procedures described by reference and brief description). The concentrations of diazinon applied to the disks in the culture media inoculated with E. coli SD-4 strain were 50%, 5%, and 0.5% dissolved in acetone. No mutant colonies or inhibition zones resulted following incubation with diazinon at 37 °C for four days. The positive control (beta-propiolactone) responded as expected producing both mutant colonies (72 to 76 for 1% and 10% respectively) and zones of inhibition (25,40 and 86 mm for the 1%, 10% and 100% concentrations respectively).

This study is UNACCEPTABLE. There is insufficient detail provided related to presentation of the results and to the number of plates per condition. The report submitted is only a summary of the study, and no metabolic activation was employed.

Study Type: Mutagenicity - Sister Chromatid Exchanges in Cultured Mammalian Cells

Accession No.:

MRID No.: 139603

Sponsor: None

Testing Laboratory: Roswell Park Memorial Institute

Date: As published - 1981 (see citation below)

Review:

The following is the abstract of the paper as it was published under the title "Induction of sister-chromatid exchanges and cell cycle delay in cultured mammalian cells treated with eight organophosphorous pesticides" by H.H. Chen, J.L. Houeh, S.R. Siriami, and C.C. Huang, as published in Mutation Research 88:307-316 (1981).

"Summary

Induction of sister-chromatid exchanges (SCE) and cell cycle delay in Chinese hamster cell line V79 after treatment with 8 organophosphorus pesticides (OPP) were studied. In addition, these effects were also studied using 1 of the 8 OPP in 2 human lymphoid cell lines. In V79 cells, 6 of the 8 OPP induced significant increase of frequencies of SCE and all the OPP induced various degrees of cell cycle delay. The 6 OPP in decreasing order of SCE induction are methyl-parathion, demeton, trichlorfon, dimethoate, malathion, and methidathion. The 2 OPP that had no effect on SCE are diazion and disyston. The extents of induced cell cycle delay are generally related to the OPP concentrations but does not necessarily correlate with the extent of induction of SCE among the OPP studied. The results of studies on the effect of methyl-parathion on SCE and cell cycle delay in 2 human cell lines showed that both lines had significant and dose-dependent increase of SCE frequencies similar to those observed in V79 cells. In contrast to V79 cells, however, cell cycle delay was not as prominent in the human lines at comparable doses. These studies indicated that 5-bromodeoxyuridine labeling for analyzing SCE and cell cycle delay is a very sensitive method in assessing mutagenic potential of environmental compounds especially those that are highly toxic to and rapidly degradable in mammalian cells such as OPP."

TB comments. This study is UNACCEPTABLE as a definitive mutagenicity study since the chemical was not tested in the presence of mammalian metabolic activation. TB notes that this negative result is in contrast to the positive response in mudminnows noted from a different laboratory.

Study Type: Mutagenicity - Sister Chromatid Exchange
(with mudminnows)

Accession No.:

MRID No.: 00142045

Sponsor: None

Testing Laboratory: Dept of Biology, Eastern Washington University

Date: 1983 (As published)

Review:

The following in the abstract of this paper as it appeared in Mutation Research 118:61-68 (1983) in the paper entitled "In vivo induction of sister-chromatid exchange in Umbra limi by the insecticides endrin, chlordane, diazinon, and guthion" by N.V. Vigfusson, E.R. Wise, C.A. Pernsteiner, and R.J. Dawson.

"Summary

Central mudminnows, Umbra limi, were exposed to the insecticides endrin, chlordane, diazinon and guthion at four concentrations ranging from 5.4×10^{-12} M to 5.4×10^{-9} M in the aquaria water. Endrin, chlordane and diazinon caused significant increase in the frequencies of SCE. The results of these tests in part are in contrast to previous work which did not find endrin to be mutagenic. Our results suggest that the in vivo SCE test is an efficacious method of detecting mutagenic pesticides in water."

Inspection of the data tables indicates that diazinon at 5.4×10^{-10} and 5.4×10^{-9} M resulted in statistically significant increases (p 0.01 level) of more than twice the negative control rate in intestinal tissue cells (higher concentrations were toxic).

TB considers this study INCONCLUSIVE as a definitive study that diazinon induces sister chromatid exchanges. Additional data and a repeat study in which diazinon is tested versus known positive controls (if available) in this system will have to be presented.

Study Type: Mutagenicity - Dominant Lethal (Mice)

Accession No.:

MRID No.:

Sponsor: Ciba-Geigy

Testing Laboratory: Ciba-Geigy - Basel, Switzerland
Experiment No. 327507

Date: March 20, 1975

Review:

Diazinon (technical purity not stated) was dissolved in an aqueous solution of carboxymethylcellulose and male mice (three groups of 12 each) were dosed with 15 or 45 mg/kg as a single dose. No positive control group was included. Each male mouse was then placed with three female mice and allowed to mate. Three fresh females were placed with each male after 1 week for 6 consecutive weeks. The 6-week interval is not coincident with the cycle of the maturation of the germ cell from a spermatogonia to the mature spermatozoon. According to Dr. I. Mauer, this interval is 8 weeks. Following mating, the females were autopsied on the 14th day of presumed pregnancy and the numbers of live embryos and embryonic deaths were noted. The uteri were also placed into a solution of ammonium sulphide in order to detect sites of early embryonic resorptions.

There were no significant differences in the mating ratio, number of implantations and embryonic deaths among the three study groups. There were some signs of reaction to treatment (dyspnea and somnolence) in the males shortly after treatment.

This study is UNACCEPTABLE. Within the limits of the study conditions diazinon is not shown to be a dominant lethal mutagen. A limitation is that the diazinon may not have reached the testes to cause an effect in the maturing sperm. Such a limitation pertains to all in vivo dominant lethal studies. The UNACCEPTABLE classification was made on the recommendation of Dr. I. Mauer who advised that the highest dose tested was not a toxic dose and that there were insufficient details on the experimental procedures, and incomplete data on clinical and/or reproductive effects.

Additional Mutagenicity Studies

[Note: These studies are considered by TB to be unacceptable as definitive mutagenicity studies because they are studies reported in the literature and several other chemicals were tested at the same time. Most of these studies lack sufficient detail to be validated on the basis of the information provided.] This is not intended to be a complete list of papers reporting studies of the mutagenicity of diazinon.

Citation

Results

R.C. Woodruff, J.P. Phillips, and D. Irwin. Pesticide-induced complete and partial chromosome loss in screens with repair defective females of Drosophila melanogaster. Environmental Mutagenesis 5:835-846 (1983)

Negative at 100 ppb (only dose tested of a formulation).

T.C. Marshall, H.W. Dorough and H. Earle Swim. Screening of Pesticides for Mutagenic Potential Using Salmonella typhimurium mutants J. Agri. Food Chem. 24:560-963 1976.

Negative with or without metabolic activation in strains of S. typhimurium (only one non toxic level tested at 1000 ug/plate).

M. Moriya, T. Ohta, K. Watanabe, T. Miyazowa, K. Kato, and Y. Shirasu. Further mutagenicity studies in pesticides in bacterial reversion assay systems. Mutation Research 116:185-216 (1983).

Diazinon was reportedly negative in S. typhimurium or E. coli tests. Only summary and qualitative results reported.

Byeon, W., Hyun, H.H., Lee, S.Y. Mutagenicity of pesticides in the "Salmonella microsomal enzyme activation system." (Translated from Korean J. Microbiology 14:128-134 [1978].)

Diazinon was reportedly negative in S. typhimurium, with and without metabolic activation. Only one dose tested and only qualitative (+/-) results reported.



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

CASWELL FILE

005206

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

JUN 23 1986

INTRABRANCH MEMORANDUM

SUBJECT: Diazinon Registration Standard: Review of a neurobehavioral study in mice (Spyker and Avery, 1977).

TO: John Doherty, Ph.D.
Section II

THRU: Jane E. Harris, Ph.D.
Section Head, Section VI

JEH 6/20/86

William Burnam
Deputy Branch Chief

FROM: William F. Sette, Ph.D.
Section VI

William F. Sette
WFS 6/23/86

Chemical: Diazinon. O,O-Diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate.

Caswell No. 342

As you requested in your memo of May 28, I have reviewed the study by Spyker and Avery (1977). In my judgment, this study provides no convincing evidence of neurotoxicity in mice following prenatal exposure to either 0.18 or 9.0 mg/kg/day during gestation. In general, the tests appear to be valid and the study is of acceptable design and conduct. Most of the effects seen were not dose dependent, the behavioral effects were not consistent with one another, and taken as a whole, the data do not readily suggest an identifiable construct of neurotoxicity, such as a central-peripheral axonopathy, where motor signs and pathology can be correlated. However, the high dose is not adequate to define frank toxicity. Although significant (14%) reductions in weight gain in the dams were seen in both groups, they were not dose dependent.

Reviewed by: William F. Set , Ph.D.
Section VI, Tox. Branch (TS-769C)
Secondary reviewer: Jane Harris, Ph.D.
Section VI, Tox. Branch (TS-769C)

005206

DATA EVALUATION REPORT

STUDY TYPE: Developmental Neurotoxicity TOX. CHEM. NO.: 342

ACCESSION NUMBER: MRID NO.:

TEST MATERIAL: Diazinon

SYNONYMS: O,O-Diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl)
phosphorothioate

STUDY NUMBER(S): Journal of Toxicology and Environmental Health
3:989-1002

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TITLE OF REPORT: Neurobehavioral Effects of Prenatal Exposure to
the Organophosphate Diazinon in Mice

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CONCLUSION: This study provides no convincing evidence of an effect of
Diazinon on the nervous system.

Core Classification: Supplementary

MATERIALS:

1. Test compound: Diazinon (technical) Lot # MG8-FL741305
2. Test animals: Species: F₂ dihybrid mice derived from
female C57BL/6X, male A/JAX and female C3H/HeX,
male BALB/c F₁ offspring.

METHODS

The test material was incorporated into peanut butter and given in 1 ml units daily at doses of 0, 0.18, or 9.0 mg/kg/day. Animals were dosed beginning on the day after first appearance of a vaginal plug and continuing until parturition. There were 3 groups of mice with 6 mice/group. The mothers were weighed daily. At birth, pups were randomly assigned to give each dam within a treatment group four male and four female pups. Animals were not cross fostered. The offspring were weighed daily and observed for the appearance of seven developmental landmarks. They were weaned on day 28 and housed in groups of six by treatment group. Thereafter, all testing was blind. The schedule of testing is given below.

<u>DAY</u>	<u>TEST</u>	<u>EFFECTS</u> (+/- statist. signif. at p<0.05)	
		0.18 mg/kg	9.0 mg/kg
38	visual cliff	-M,+F	-
	auditory startle	-	-
	ammonia withdrawal	-	-
50	swimming measures	-	+ activity increases
60	rod clinging test	+	+ increased cling time
65	rotarod (3 trials)	-	- dose-dependent decreases in endurance
70	inclined plane	+	+ decreased angle grip
75-76	open field activity	-	-
87	Maze performance deprivation to 80% of free-feeding weight		
T1*	10 training trials		
T2	10 training trials		
P1	5 test trials	-	-
P2	7 test trials	-	-
P3-P5	11 test trials	-	+ decreased speed on last day (P5) only
110	All mice were sacrificed, brains removed, fixed in Bouin's solution, washed in lithium carbonate, dehydrated, embedded in paraffin, and 7 μ m serial sagittal sections prepared. Hematoxylin and Eosin stain was used.		

* The precise dates of training and performance were not given; relative dates are indicated.

RESULTS

Both groups of exposed mice gained significantly less weight (14%) during pregnancy but dose groups were not different from each other. Low dose mothers had a smaller litter size than controls or high dose animals. The rate of weight gain for the high dose pups was less for at least the first 3 weeks of life.

For the developmental landmarks, the only effects seen were in low dose mice and involved a roughly 2 day delay in the appearance of a contact placing response and appearance of external genitalia.

The behavioral results are summarized in the table above, listing the statistically significant effects.

High dose mice showed an increase in swimming activity and decreased speed of running in the maze on the last day of testing. High and low dose mice showed an increased time of clinging on a rod, and a decrease in the angle of an inclined plane they could hang on. On the rotarod, activity decreased from a time of 1,006 +/- 621 secs for controls to 407 +/- 161 secs for low dose mice to 103 +/- 56 secs for high dose mice. While the authors note that these were not statistically significant, they do show a dose-dependent effect. For rod clinging and the inclined plane, there were essentially no differences between dose groups.

The neuropathological data revealed dense aggregations of chromatin-containing cells in the forebrains of 5 of 8 mice examined in the high dose group. No such effects were seen in low dose or control brains.

Evaluation

In general, the data seem to be based on valid study conduct.

Because this is a published study from the open literature, all the data were not available for independent evaluation.

But there are a number of inconsistencies in the reporting of the data, e.g., in Figure 2, the mean body weights of all 3 groups appear to overlap on week 14, in contrast to the assertion in the figure legend that the high dose mice were still different.

Further, it is difficult to form a construct or hypothesis about the effects seen that is consistent with all the data. Specifically, mice show an increase in time they cling to a rod, but a decrease in the angle of a plane that they can cling to. While swimming activity increased, rotarod activity decreased. Running speed in the maze decreased, but only on the last day of testing. Open field activity was unchanged. At best, there is an apparent decrease in balance. The neuropathological data may be regarded in the same way. That is, the effects appear real, but the functional significance of these changes or the relation

of changes in the forebrain to the observed motor deficits is unclear. Thus, the data are valid but limited, due to the lack of dose dependent data and our inability to make a coherent hypothesis of their neurological cause or toxicological significance.

Last, while the 2 doses span a range of 50, it is not clear that the high dose provides unequivocal evidence of toxicity. While both doses produced 14% reductions in dam weight gain, these effects also were not dose dependent. The only related data I am aware of (although I do not follow this literature and have not done a search) indicates that weanling (28 day old) rats showed significant decreases in activity following 50 mg/kg of Diazinon while for adult (60 day old) rats the decreasing effects of 100mg/kg but not 50mg/kg were statistically significant (U.S.EPA, 1985). Thus, younger animals (and perhaps the fetus) may be more sensitive to Diazinon. It should be noted that the EPA study did not find significant differences in LD₅₀s or plasma and brain cholinesterase. Both groups showed significant decreases in serum cholinesterase, and adults recovered more slowly.

I conclude that this study provides no convincing evidence of neurotoxicity following prenatal exposure to Diazinon.

Reference

U.S. Environmental Protection Agency. 1985. Age-related neurotoxic effects of pesticides: Youth-in-agriculture research. Unpublished report. S. Padilla, R. MacPhail, L.Reiter. Task # 51595E104.