11-29-77

EEB BRANCH REVIEW

DATE: IN ____ OUT ____ IN 4/4/77 OUT 11/29/77 IN ____ OUT ____

FISH & WILDLIFE ENVIRONMENTAL CHEMISTRY EFFICACY

FILE OR REG. NO. 201-274

PETITION OR EXP. PERMIT NO. 7F1918

DATE DIV. RECEIVED 2/3/77

DATE OF SUBMISSION

DATE SUBMISSION ACCEPTED 3/3/77 3CID-2B-Yes

TYPE PRODUCT(S): (I) D, H, F, N, R, S

PRODUCT MGR. NO. F. gee (16)

PRODUCT NAME(S) Bidren 8

COMPANY NAME Shell Chemical Co.

SUBMISSION PURPOSE Use on Citrus (oragges, grapefruit, lemons, in California and Arizona)

CHEMICAL & FORMULATION Dimethyl phosphate of 3-hydroxy N, N-dimethyl-cis-crotonamide (Bidren)

1.0 Introduction

1.1 This is a review of Shell's data for the registration of an end-use Bidren product; BIDREN-8 (201-274) is proposed for use on citrus.

1.2 Shell's letter (2/1/77) references their PR 70-15 response in support of the proposed use: "Environmental Impact Statement - BIDREN" submitted 10 Feb. 72 (#094598).

1.2.1 The following referenced studies are not germane to the EC Review.

Quarterly Progress Report USDI Fish and Wildlife Service, Florida. "Effects of Pesticides - Chemical Assays", October 1, December 31, 1963. Tab #18.

T. E. Shellenberger and C. W. Newell. "Acute and Subacute Toxicity and Cholinesterase Studies of Shell Compound SD 3562". Stanford Research Institute, January 31, 1962. Tab #19.

Robert L. Doyle and John R. Elsea. "Repeated Application of Technical BIDRIN Insecticide and AZODRIN to the Skin of Rabbits", Hill Top Research, Inc., Miamiville, Ohio, August 17, 1965. Tab #20.

Letter dated March 28, 1967 from Dr. T. E. Shellenberger, Gulf South Research Institute to Dr. Ralph F. Glasser, Shell Chemical Company, Re: "Toxicological Evaluation of Bobwhite and Japanese Quail with AZODRIN and BIDRIN". Tab #21.

T. E. Shellenberger, G. W. Newell, R. F. Adams and J. Barbaccia. "Cholinesterase Inhibition and Toxicologic Evaluation of Two Organophosphate Pesticides in Japanese Quail", Toxicol. App. Pharmacol 8, 22, 28 (1966). Tab #22.

Lewis S. Schanker. "Mechanisms of Drug Absorption and Distribution". Annual Reviews Pharmacology Vol. 1 pg. 29 (1969). Tab #23.

Corwin Hensch. "A Qualitative Approach to Biochemical Structure - Activity Relationships", Accounts of Chemical Research Vol. 2 Pg. 232-239 (1969). Tab #24.

Letter dated November 3, 1965, from Mr. Oliver B. Cope, USDI, to Mr. M. J. Sloan, Shell Chemical Co., Re: "Toxicological Evaluation of Bluegill Sunfish with BIDRIN". Tab #27.

Informal Report: Toxicity of SD 3562 (BIDRIN) to Gambusia fish (1962). Included in Proposed "No Residue" Label Application for Use of BIDRIN on Cotton - January 1963. Tab #28.

Annual Progress Report, Denver Wildlife Research Center (1966). Tab #29.

Robert L. Doyle and John R. Elsea, "Toxicity Studies on BIDRIN in Geese", Hilltop Research, Inc., Miamiville, Ohio, December 11, 1963. Tab #30.

C. M. Menzie. "Metabolism of Pesticides". Bureau of Sport Fisheries and Wildlife - Special Scientific Report - Wildlife No. 127 Pg. 55, Washington, D. C. (1969). Tab #11.

J. W. Barnett and B. W. Arthur. "Absorption, Transloccation, and Degradation of BIDRIN by Cotton, Peanut, and Soybean Plants." Unpublished paper (1962). Tab #12.

D. L. Bull and D. A. Lindquist. "Absorption and Metabolism by Boll Weevils, Bollworms and Cotton Plants of BIDRIN" from Shell Chemical Company (1962). Tab #13.

1.2.2 The following referenced studies contain EC data which was reviewed (5/75), and will not be validated at this time. Refer to Dr. Rogoff's memo to Mr. Compt of August 12, 1977.

Project Progress Report FD/5/65. "The Stability of BIDREN in Soil", Woodstock Agricultural Research Centre - "Shell" Research Limited, February, 1965. Tab #1.

Project Progress Report FD/23/65. "The Development of BIDRIN Formulations III. Studies of Granules for Use in Soil", Woodstock Agricultural Research Centre - Shell Research Limited, June 1965. Tab #2.

W. E. Hall and Yun-Pei Sun. "Mechanism of Detoxication and Synergism of BIDRIN Insecticide in House Flies and Soil". J. Econ. Ent. 58 (5) pg. 845 (1965). Tab #3.

Project Progress Report FD/48/65. "The Adsorption and Decomposition of BIDRIN and AZODRIN in Soil", Woodstock Agricultural Research Centre - Shell Research Limited, November 1966. Tab #4.

Residue Data for Soil. Tab #6.

K. E. Elgar and I. A. MacDonald. "Analysis of Crops for Residues of BIDRIN and Its Metabolites". J. Sci. Fd. Agric. 17 pg. 500 (1966). Tab #7.

D. L. Bull and D. A. Lindquist. "Metabolism of 3-hydroxy-N, N-dimethyl-crotonamide Dimethyl Phosphate by Cotton Plants, Insects, and Rats". J. Agric. Food Chem. 12, No. 4 pg. 310 (1964). Tab. #8.

R. E. Menzer and J. E. Casida. "Nature of Toxic Metabolites Formed in Mammals, Insects and Plants from 3-(Dimethoxyphosphinyloxy)-N,N-dimethyl cis-crotonamide and Its N-Methyl Analog". J. Agr. Food Chem. 13 No. 2 pg. 102 (1965). Tab #9.

R. E. Menzer and W. C. Dauterman. "Metabolism of Some Organophosphorus Insecticides". J. Agr. Food Chem., 18 No. 6 pg. 1031 (1970). Tab #10.

Bioassay Card Biological Science Research Center, Modesto, California, August, 1964. Tab #14.

N. P. H. Brown, A. J. Forster and C. G. L. Furmidge. "Stability of Agricultural Chemicals. I - Hydrolytic and Thermal Stabilities of Phosphorylated Crontonamides". J. Sci. Fd. Agric., 17 pg. 510 (1966). Tab #17.

2.0 Directions for Use

Oranges, grapefruit and lemons in California and Arizona.

2.1 Rates

16 fluid oz. (1.04 lb ai)/acre. Applied as a spray in 200-300 gal/acre or in 10-20 gallon, by air.

2.2 Restrictions

"...Do not make more than 2 applications per growing season... Do not apply to groves with standing water, to head ditches containing water or to irrigation ponds...Do not apply after July 1..."

2.3 Precautions

"...Keep out of lakes, ponds and streams. Use only as directed on this label. Note use restrictions for citrus in California and Arizona..."

2.4 Disposal

"...Do not reuse containers for any purpose. Decontaminate containers by rinsing thoroughly with water and alkaline detergent. Destroy containers by breaking and burying fragments in isolated area. Dispose of rinsings in a way so as not to constitute a hazard, or contaminate water supplies."

3.0 Discussion of Data (Not previously rev'd)

3.1 Fish Accumulation (submitted 2/2/77).

"Bio-accumulation of Bidrin Insecticide in Fish - Residue levels of Bidrin and two metabolites (SD-12210 and SD-9129) in Rainbow Trout resulting from the exposure to 0.5 ppm Bidrin in water"; Report TIR-24-633-76-B.

The trout [21/Arg ca 45 gms] in this study were maintained (56°F) in aerated water (135-liter) containing Bidrin (ca 0.5 ppm); at intervals of 3 to 4 days the fish were transferred to a freshly prepared solution of Bidren. The testing included exposure (31-days) and depuration (17 days). Samples of live whole fish and water were preiodically sampled for analysis (GLC-AFID). The included methodology indicates a sensitivity (MDC) in water and tissue of 0.01 ppm for both Bidren and its degradate SD-9129 (Azodrin); and, 0.02-0.03 ppm for SD-12210 (compound II). The recoveries (whole trout spiked with Bidren 0.05 ppm; Azodrin 0.08 ppm. Arg'd for Bidren 93% and Azodrin 105%. Compound II is relatively unstable (recovery ca 79%) and so its glycoside was used as the standard in the determination of compound II.

Summary of Rainbow Trout Data

Residues (ppm)

Day	Water Bidren(Arg)	Whole fish residues Bidren	SD-9129	SD-12210
0	0.49	<0.01	<0.01	<0.02
10	0.49	<0.01	<0.01	<0.02
20	0.49	0.01	<0.01	<0.02
25	0.50	0.01	<0.01	<0.03
31	0.44	0.01	<0.01	<0.03
Dupuration				
1	-	<0.01	<0.01	-
4	-	<0.01	<0.01	-
12	-	<0.01	<0.01	-

Conclusion

Data deficiencies

(a) The reported fish accumulation data are limited to parent Bidren and two of its degradates, compound II and III. If degradates in addition to II and III resulted from either photodegradation or soil metabolism of Bidren additional trout accumulation data may be needed. The submitted hydrolysis data indicates Bidren stable, to hydrolysis.

Since the fish study was not radiolabelled its assessment will not be possible without the following data:

(1) Photodegradation (see 5.4.2); the identity of the soil and water degradates have not been submitted and are needed (4.2).

(2) Soil metabolism (Sec. 5.4.3); the identity of the aerobic degradates have not been submitted and are needed.

(3) Leaching (sec 5.4.6); the leaching properties of the unidentified soil degradates have not been submitted are are needed.

(b) After the above data have been submitted, the acceptability of the submitted fish accumulation data and the need for additional data can be determined.

The data indicates:

(a) Bidren and degradates II and III do not bioaccumulate in trout significantly (< 0.01 ppm). Recoveries may have been much less than the reported 93-105%. However, assuming recoveries of only 1% to 2% the accumulation factor would be 1 to 2 instead of 0.02.

(b) A catfish accumulation study (static system) with radio-labeled Bidren. See sect. 5.4.8, may be needed.

Data on fish accumulation are required.

3.2 Effects on Nematodes

Primary Screening - BAS 3562, Nematodes - in Water, Biological Science Research Center, Modesto, California, April, 1955; Acc 094518, Tab #15.

Primary Screening - SD 3562 - Nematodes in Soil, Bioligical Science Research Center, Modesto, California, November, 1969; Acc 094598, Tab #16.

Also, summary and conclusion sections, pg. 11-12.

Data not previously reviewed:

Both Tabs #15 and #16 consists of single laboratory work sheets. Shell's comments on the tests are in the summary. Bidren in soil (35 ppm) increased the mortality rate of root-knot nematodes 29% within a 4-week period.

Conclusion

Nematode mortality is increased by soil residues of Bidren.

The data is ancillary.

3.3 Bidren and its reported degradates, by degradation site.

Degradates (sec. 3.4) Name or Code	Soil[XX]	Water	Plant	Animal
Compound I or SD-3562		Hydrolysis (stable)	Cotton (c) Soybean(S) Peanut (P)	Rat (R) Mice (M) Rabbit (Rab.) Dog (D) Goat (G)
Compound II or SD-12210			C,S,P	R,M,Rab., D, G
Compound II or SD-9129 or Azodrin			C,S,P	R, M, Rab, D, G
Note (1)				R, M, Rab., D, G
Note (2)				Rab, D, G
Compound IV or dimethy phosphate		Hydrolysis (H)	C	Boll Weevil
Compound V or desmethyl Bidren		H	C	Boll Weevil
Note (3)		H		
Bidren Acid			C	
Compound VII			C	
Compound VIII			C	
Note (4)		H		
Compound IX		H	C	Boll Weevil
Methanol		H		R, M, Rab, D, G
Acetone		H		
Carbon dioxide		H		
Phosphate			C	Boll Weevil
Unknown A			C	
Unknown B			C	
Unextractable (Bound) activity			C	

Water soluble metabolites not identified		C	R, M, Rab, D, G

3.4 Table of chemical names for the Bidren degradates by Shell's name(s) and code number(s). Also, the degradates which have not been coded (compounds noted #1 thru #4 of table 3.3) are included.

Compound	Names, Chemical and Code
I	Bidren: cis-and trans (alpha & beta)[15.1]. Dimethyl phosphate of 3-hydroxy N, N-dimethyl-cis-crotonamide.
II	Dimethyl phosphate of 3-hydroxy-N-hydroxymethyl-N-methyl, cis crotonamide. Also "hydroxymethyl Bidren"
III	Dimethyl phosphate of 3-hydroxy-N-methyl cis-crotomamide. Also "Nemethyl Bidren", or Azodrin.
IV	Dimethyl phosphate
V	Methyl hydrogenphosphate of 3-hydroxy-N,N-dimethyl crotonamide. Also "Desmethyl Bidren"
VI	Dimethyl phosphate of 3-hydroxy crotonic acid. Also "Bidren acid"
VII	Methyl hydrogen phosphate of 3-hydroxy crotonic acid. Also "Desmethyl Bidren Acid"
VIII	Dimethyl phosphate of N-(glycosyloxymethyl)-3-hydroxy-N-methyl-cis-crotomamide. Also "Sugar conjugates of compound V"
IX	Methyl phosphate

Compound Names, Chemical and Code

Noted (1) Dimethyl phosphate of 3-hydroxy-N-hydroxy-N-methyl-cis-crotomamide.

Or "N-hydroxy methyl Azodrin"

Or N-methyl-N-hydroxy methylamide of Bidren.

Noted (2) Dimethyl phosphate of 3-hydroxy-cis-crotomamide.

Or Unsubstituted amids of Bidren.

Or N-dimethyl Azodrin.

Noted (3) N, N-dimethylacetoacetamide (Also, N-mono)

Noted (4) Di-methylamine (also mono)

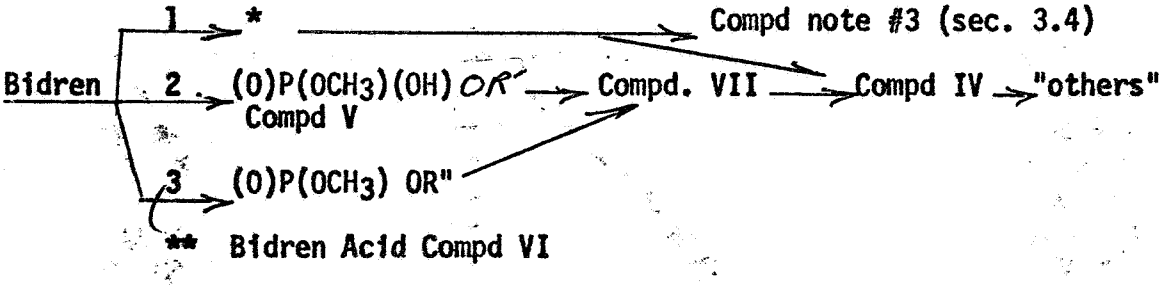
XX None of the soil degradates or photodegradates have been identified.

3.5 Bidren Degradation

The degradation may proceed through either of three pathways; additional details are shown on the attached Sheet M. For the chemical name of Compounds IV, V, etc., see section 3.4; the sites of degradation are given, see section 3.3.

Showing Bidren -

$(O)P(OCH_3)_2[OCCH_3{:}CHC(O)OH]$ as $(O)P(OCH_3)_2$ OR'



* Identified only as a hydrolysis reaction; soil and photodegradates have not been identified.

** Four plant and animal metabolites (see attached sheet M) are identified as intermediats to the formation of compound VI.

"Others"; include mono-methyl phosphate (LX), phosphate, acetone, methanol, carbon dioxide.

Conclusion

4.1 Hydrolysis (Rev'd 5/75)

The hydrolytic halflife of Bidren at 20°C exceeds 100 days; data is given at pH's 1 and 9, and is sufficient to interpolate an equivalent stability at pH's 5 and 7.

Hydrolysis is somewhat faster in alkaline than in acid solution, and the degradation is more extensive.

Acid degradates: methyl phosphoric acid; N, N-dimethylacetamide (also N-mono-) and small amounts of acetone, methanol, and carbon dioxide.

Alkaline degradates: dimethyl phosphate, acetone, carbon dioxide, methanol, and di-methylamine (also mono-).

Degradates were obtained in sufficient amount for characterization by rising both the temperature (>50%) and concentration (> 38m moles/L) of the Bidren solutions. The data is adequate.

4.2 Photolysis (Rev'd 5/75)

Ancillary: Bidren on glass is stable to photodegradation. Data is needed on soil and in water.

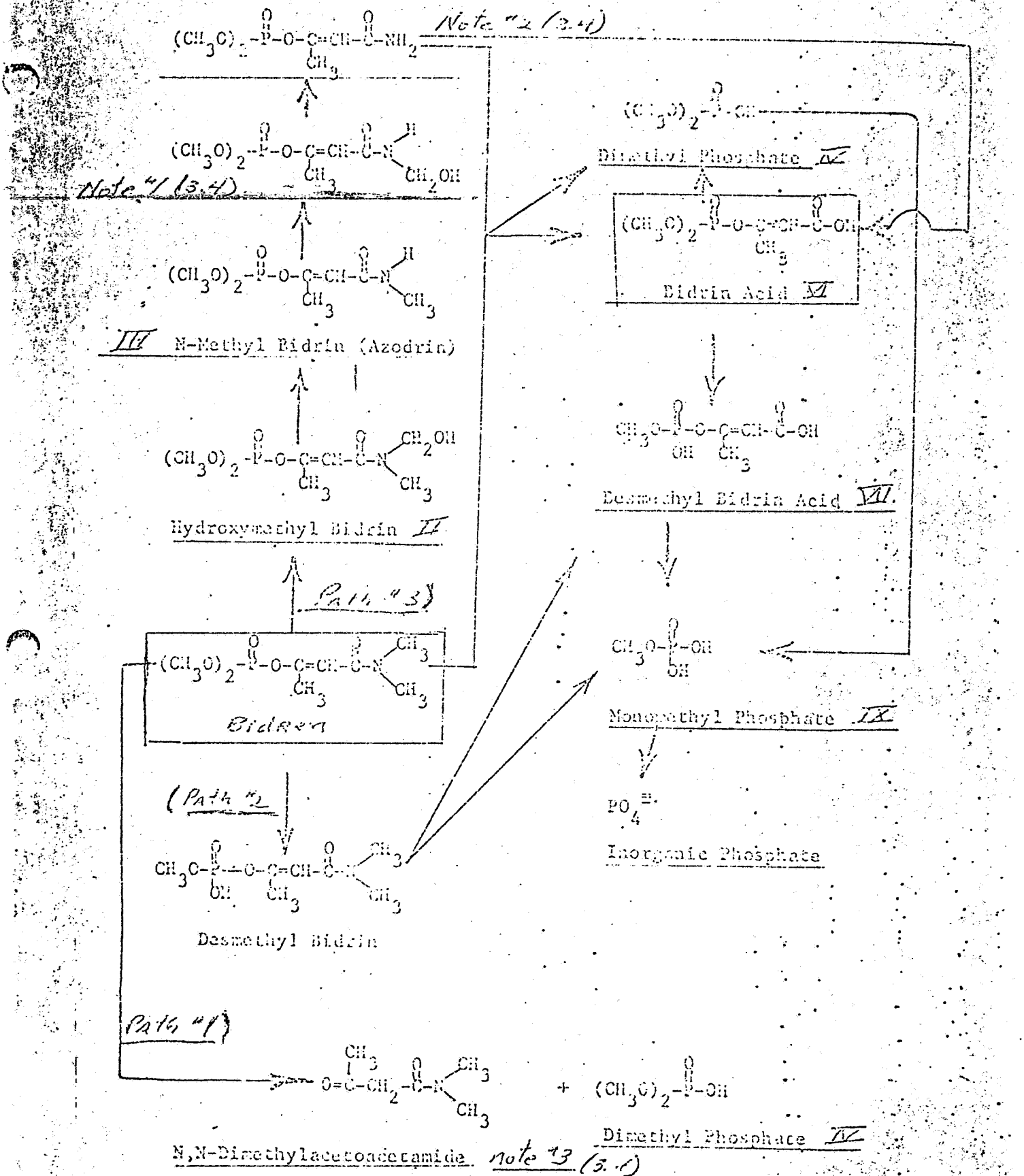
4.3 Soil Metabolism (Rev'd 5/75)

Under aerobic conditions Bidren's halflife is less than 6 days. The identity of the degradate, none were identified, is needed.

4.4 Microbial metabolism (Rev'd 5/75)

(a) Effects on pesticide: Bidren's increased stability on a sterile versus a fresh loam soil (negligible at 30 days versus a T 1/2 ~7 days) indicates degradation ~~is~~ was

Attachment IV



essentially microbial. The identity of the degradating organisms is needed.

Ancillary: The presence of sodium azide (Tab #4) or drying (Tab #2) increased Bidren's soil stability.

(b) Effect on microbes: Organisms tested were not important to either soil fertility or soil degradation processes. Data showing Bidren's effects on appropriate microbes are needed.

Ancillary(3.2): An increase of 29% occurred in the mortality of nematodes exposed to Bidren residues of 35 ppm, during a 4 week period.

4.5 Leaching (Rev'd 5/75)

Bidren leaches readily, 25 mls of water leached through the soil of a greenhouse pot. Data is needed on soils (4), by a standardized column procedure. Aged-leaching data is also needed, none has been submitted.

4.6 Field dissipation (Rev'd 5/75)

Parent-Bidren residues dissipated rapidly (T 1/2 ≤ 1 week) following a direct application to an Arkansas soil, previously planted to cotton. The report is not relevant to the proposed use on citrus. Data is needed under use conditions, from four citrus areas.

4.7 Fish accumulation (3.1)

(a) Catfish accumulation under static conditions have not been submitted for Bidren and are needed.

(b) Residues of parent Bidren and compounds II and III (see 3.4) were determined in Rainbow Trout (whole) by GLC. The exposure (0.5 ppm/31 days) indicates an accumulation factor < 2.0.

However, the study was not done with 14C-Bidren; and data has not been submitted establishing degradates II and III as the only degradates likely to accumulate. Therefore, to determine both the acceptability of the trout data and the need for additional fish-data we will need photodegradation (see 5.4.2), soil metabolism (5.4.3) and leaching (5.4.6) studies. These studies are normally required in support of a tree fruit-nut use and have not been submitted.

4.8 Metabolism

The Bidren metabolites in orange (< 0.01 ppm) have not been identified. The following degradates of Bidren have been identified. (See also section 3.3).

Shell's Codes	Chemical
Compound I SD-3562	Dimethyl phosphate of 3-hydroxy-N,N-dimethyl-cis-crotonamide cis/trans ratio 15/1 (also termed alpha/beta ratio.
Bidren	Rel. stable to hydrolysis. Found in cotton, soybean, peanut; rat, mice, rabbit, dog, goat.
Compount II SD-12210	Dimethyl phosphate of 3-dydroxy-N-hydroxymethyl-N-methyl, cis crotonamide (also hydroxymethyl Bidren) Found in cotton, soybean; rat, mouse, rabbit, dog.
Compound III SD-9129 Azodrin	Dimethyl phosphate of 3-hydroxy-N-methyl-cis-crotomamide (Also; N-methyl Bidren). Found in cotton, soybean, peanut; rat, mouse, rabbit, dog.
(Note #1)	Dimethyl phosphate of 3-hydroxy-N-hydroxy-N-methyl-cis-crotomamide. (Also; N-hydroxy methyl azodrin; and N-methyl-N-hydroxy methylamide of Bidren). Found in rat, mouse, dog, rabbit, goat.
(Note #2)	Dimethyl phosphate of 3-hydroxy-cis-crotonamide. (Also; unsubstituted amide of Bidren and; N-demethyl azodrin). Found in rabbit, dog and goat.
Compound IV	Dimethyl phosphate. Found in cotton; bollweevil, (hydrolysis).
Compound V	Methyl hydrogen phosphate of 3-hydroxy-N,N-dimethyl crotonamide (also; desmethyl Bidren). Found in cotton and Boll weevil.
Compound VI	Dimethyl phosphate of 3-hydroxy crotonic acid (also Bidren acid). Found in cotton.

(Note #3)	N,N-dimethylacetoacetamide (also N-mono-) hydolytic products.
Compound VII	Methyl hydrogen phosphate of 3-hydroxy crotonic acid. (Also; desmethyl Bidren acid.) Found in cotton.
Compound VIII	Dimethyl phosphate of N-(glycosyloxymethyl)-3-hydroxy-N-methyl-cis-crotonamide. (Also; sugar conjugate of Compound II). Found in cotton.
(Note #4)	Dimethylamine (also mono-), hydrolytic products.
Compound IX	Methyl phosphate Hydrolytic product. Found in cotton and Boll weevil.
Methanol Acetone Carbon dioxide	Hydrolytic products; found in rat, mouse, rabbit, dog.

5.0 Recommendations

5.1 No opinion is given.

5.2 The following environmental chemistry data gaps* are noted for the proposed use of Bidren on citrus.

(1) Photodegradation on soil.

(2) Photodegradation in water.

* A description of the data required for the registration of fruit-crop uses follows in section 5.4.

5.3 The following studies are not acceptable; their deficiencies are listed.

5.3.1 Aerobic Soil Metabolism (Item 04-01)

Data is needed which identifies the Bidren soil degradates which exceed 10% of the applied, none have been identified. Both tables and graphs of the data are needed which show the formation-decline pattern of the degradates; see section 5.4.3.

5.3.2 Microbial Metabolsim

A. Effects of microorganisms on Bidren (Items 04-01 and 04-34).

Data on the identity of the soil organisms responsible for the reported degradation of Bidren are needed, see section 5.4.4.

B. Effect of Bidren on Microbes (Items 04-30).

Data is needed which show Bidren's effects in the soil microbes which are related to soil fertility and normal soil degradation processes; see Section 5.4.5.

5.3.3 Leaching (Item No. 04-09)

The leaching-pot data, the only leaching data submitted, is not adequate. Leaching data are needed which will provide a quantative measure of the leaching properties of Bidren in the environment. Use a standard column-leaching protocol, radioisotopic or comparable technique, and provide data on four soils. Aged-leaching data are also needed, none has been submitted; see Section 5.4.6.

5.3.4 Field Dissipation (Item 04-10)

Data is needed under actual use conditions from four citrus growing areas; see Section 5.4.7.

5.3.5 A catfish accumulation study conducted under static conditions, using a radioisotopic technique may be required.

A. Since the trout study (TIR-24-633-76-B) was not conducted with radiolabelled Bidren, and because of other data gaps, we cannot determine whether the data is acceptable. We will need the following data to assess the study.

(1) A photodegradation study, see section 5.4.2, which includes the identity of the soil and water photodegradation products.

(2) An aerobic soil metabolism study which includes the identity of the soil degradates; see section 5.4.2.

(3) A leaching study, which provides data on the leaching properties of both Bidren and its degradates; see section 5.4.6.

B. The chemical will be used in areas where leaching is likely to occur. If degradation occurs in 1, 2, and 3 (see A); or if degradates leaches, then a catfish study will be needed. The acceptability of the trout study, and the need for additional fish accumulation data, can be determined when data are submitted for review. Note that the submitted hydrolysis data is acceptable, Bidren is considered stable to hydrolysis.

5.4 The environmental chemistry data required to support the proposed use on citrus are listed in the tree fruit-nut column of the attached sheet. Acceptable protocols for obtaining the data follow:

5.4.1 Hydrolysis

Hydrolysis data are required for all pesticides. Studies are conducted in darkness using radioisotopic or other comparable techniques at different pH values (acedic, neutral and basic) at two concentrations and two temperatures. Aliquots in duplicate should be taken at four sampling time intervals, with at least one observation made after one-half of the pesticide is hydrolyzed, or thirty days, whichever is shorter. A material balance (the total accountability at the completion of an experiment of the pesticide introduced into a defined system including both identified and unidentified products) half-life estimate, and identification of degradation products for the pesticide must be provided. Studies utilizing distilled water provide an upper limit estimate for persistence of pesticides in the aquatic environment. Hydrolysis in natural waters may be carried out to supplement studies in distilled water.

5.4.2 Photolysis

Photodegradation studies in water are required for all terrestrial uses. Studies in soil are required for crop uses. Conduct photodegradation studies using radioisotopic or comparable techniques at one concentration under natural or simulated (greater than 280 nm 280 X 10^{-9} meters wavelength)

sunlight. Such studies must provide material balance, half-life estimate and the identification of photoproducts. Rate studies are conducted in distilled or deionized water and sampling should continue until twenty percent degradation is observed, and for thirty days to identify photoproducts. Yield of photoproducts may be increased by changing such conditions as wavelengths, concentration, photosensitizers and solvents other than water. Supplemental rate and photoproduct studies may be carried out in natural water for aquaric uses. Studies performed on the soil used in the soil metabolism studies are preferred but other soil textures will be acceptable. The intensity of incident sunlight and time of exposure must be reported if sunlight is used as a source.

Photodegradation data must be supported by incident light intensity and percent transmission. Values for intensity in candles and lambert units are required for artificial light sources. Lattitude, time of year, atmospheric cover, and other major variables which affect incident light are to be reported when natural sunlight is used.

5.4.3 Aerobic soil metabolism

These studies determine the rate, type and degree of metabolism of the pesticide residue in a sandy loam, loam, silt loam, or other textured soil appropriate to the intended application sites. Radiolabeling in one or more positions in the pesticide molecule is required to assure adequate coverage of chemical transformations. Where radiolabeling will be of little benefit, comparable techniques are required. Residues comprising more than ten percent of initial application or 0.01 ppm should be identified. A material balance, including nonextractable residues, must be provided. The experimental dose rate must approximate field application rate. Treated soil must be maintained at temperatures of 18 to 30°C at or below 75% of 0.33 bar moisture. Collect data until a ninety percent loss of the pesticide occurs and until patterns of formation and decline of metabolic products are established. Preferred sampling times are at pretreatment; 0, 1, 2, and 7 days, 2 and 3 weeks and 1, 2, 3, 4, 6, 9, and 12 months. The study need not be conducted for more than one year for terrestrial crop and noncrop uses.

5.4.4 Effects of Mictobes on Pesticides

Impact of microbes on pesticide tranformation include comparisons of metabolic processes under sterile and nonsterile conditions during a thirty-day period. Preferred sampling intervals are 1, 3, 7, 14 and 20 and 30 days, but other intervals may be appropriate.

Acceptable soil sterilization methods are heat or high energy ionizing radiation. Attempts should be made to identify organisms responsible for degradation. For organisms which are difficult to identify, family names will be sufficient. Isolates that cannot be identified to family level must have descriptive characteristics which can be substituted for generic classification. Alternately, studies utilizing pure or defined and characterized mixed cultures of bacteria, algae and/or fungi are adequate.

5.4.5 Effects of Pesticides on Microbes

Data on effects of pesticides on microbes are obtained from studies of effects on microbial functions or microbial populations. Studies of effects on microbial function constitute a more direct approach, and are preferred to studies of effects on populations. Some effects cannot be measured directly and population studies may be the only recourse. When the functional approach is chosen, the effects on nitrogen fixation, nitrification, cellulose, starch and protein degradation are required. When the population approach is chosen, effects on pure or mixed culture populations of representative microorganisms from soil or water or obtained from culture collections are required. Appropriate organisms include free-living nitrogen-fixing bacteria and blue-green algae such as Azobacter, Colostridium, Cytophaga, Streptomyces, Pennicillium, Flavobacterium, Trichoderma, Asperigillus, Chaetomium, and Fusarium.

Animal or plant pathogens and indicators of fecal pollution are unsuitable.

Information on organism identity and media must be supplied. Organisms used as indicators must be identified by Linnaian name as well as common name. Cultures of microorganisms obtained from collections must also be identified by collection code numbers; other souces of microorganisms must be described.

Photographic evidence for claimed pure cultures not derived from collections must be submitted. Standard maintenance and test media must be identified and other media identified and described.

5.4.6 Leaching

Leaching through soil is dependent upon pesticide formulation, physical and chemical properties of pesticide and soil and environmental conditions. Add pesticide to soil(s) corresponding to the highest recommended rate for a single application and study leaching using radioisotopic or comparable techniques to provide a quantitative estimate of mobility in soil. Each study will include soils as sand (agricultural), sandy loam, silt loam, clay or clay loam having a pH range of 4 to 8 with at least one soil having an organic matter content less than one percent. Use a minimum of four soils to study pesticide leaching and elute each immediately with equivalent twenty acre-inches water. Use one of the above soils to study leaching of pesticide residues wherein the pesticide is aged in soil under aerobic conditions for thirty days prior to eluting with the equivalent of one-half acre-inches water per day for forty-five days. Two basic techniques for measuring leaching are soil column and soil thin-layer chromatography (soil TLC).

5.4.7 Field dissipation

A field dissipation study under actual use conditions is required to define the duration of potential hazards. Dissipation may decrease potential hazard of reentry into the treated area, residues in rotational crops, residues in the food web and loss of usable land and water resources through degradation processes in the treated area, or may increase potential hazards in nontreated areas through mobility. Continue analyses until a ninety percent loss of the pesticide occurs or until patterns of formation and decline of degradation products are established, or to the maximum time specified below. Sampling times include preapplication, day of application, and shortly postapplication for each single or multiple application. Succeeding samples are dependent upon degradation and metabolism characteristics and potential for reentry. Identification of residues comprising more than ten percent of initial application or 0.01 ppm is needed for the registrant to construct decline curves of residues in soil.

Fruit and nut crop uses: Soil samples are taken in increments to a depth of 12 inches from sites in four agricultural use areas for a maximum test duration of twelve months.

5.4.8 Fish Accumulation

Accumulation of residues in nontarget aquatic organisms is an indication of contamination of the food web.

Radioisotopic or comparable techniques are employed. Two exposure systems are requried: Flow-through (with constant concentration of aqueous solution of pesticide) and static (with ambient concentration of residues from treated soil). Bluegill sunfish are preferred in flow-through and catfish are required in the static system.

For the static system, sandy loam soil is treated at use rate and aged under aerobic conditions for two to four weeks prior to initiation of fish exposure. Exposure duration is 30 days with sampling at 0, 1, 3, 7, 10,14, 22, and 30 days of exposures.

Fish and water samples are taken on 0, 1, 3, 7, 10 and 14 days of depuration. Soil and water samples are also obtained prior to fish exposure interval. The amount and identity of the residue is determined for water, soil, whole body fish, edible tissue and viscera or carcass at each sample interval.

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