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

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MEMORANDUM

FILE COPY AUG 18 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: The Review of Toxicology Data in Support of the Registration of Baythroid 240 Ornamental Pyrethroid Insecticide Tempo 2.

EPA No. 3125-GLE
Record No. 168966

Project No. 1356
Tox. Chem. No. 266E

TO: George LaRocca (PM Team #15)
Registration Division (TS-767c)

FROM: John E. Whalan, D.A.B.T., Toxicologist
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

John E Whalan
8-12-86

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769c)

Budd 8/13/86

The Registrant, Mobay Chemical Corporation, has submitted toxicology data in support of the registration of Baythroid® 240 Ornamental Pyrethroid Insecticide Tempo 2. These data were submitted in response to a Toxicology Branch memorandum (John Whalan, EPA No. 3125-GLE, March 12, 1985) regarding the registration of Baythroid 240 Ornamental Pyrethroid Insecticide for commercial use in nurseries, yards, ornamental gardens, and greenhouses. It was to be applied by spraying onto trees, shrubs, flowers, evergreens, foliage plants, and adjacent areas for the control of insects. The Registrant did not indicate in the current action whether the use pattern has changed. Presumably, it has not.

Five test articles or formulations were tested in these studies:

1. FCR 1272 - technical cyfluthrin

2. Baythroid C 2 EC (Batch No. 5-03-0070)
Baythroid technical (cyfluthrin) 24.3%
[redacted]
100.0%

3. Baythroid C 2 EC (Batch No. 83-R-68-34C)
Baythroid technical (cyfluthrin) 24.3%
[redacted]
100.0%

CONFIDENTIAL

INERT INGREDIENT INFORMATION IS NOT INCLUDED

PRODUCT IMPURITY INFO IS NOT INCLUDED

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PRODUCT IMPURITY INFO IS NOT INCLUDED

CONFIDENTIAL

4. Baythroid 240 EC Formulation C - This formulation was not identified in the submission. Glenn Brussell, Mobay's Manager of Registration Research and Development informed Christine Dively (Registration Branch) that Baythroid 240 EC Formulation C is identical to Baythroid C 2 EC. He is submitting a Confidential Statement of Formulation.

5. [redacted]

There have been frequent name changes for many of Mobay's formulations without notification of the Environmental Protection Agency. Toxicology Branch has reviewed the formulation studies on the understanding that Baythroid C 2 EC and Baythroid 240 EC Formulation C are nearly identical to Baythroid 240 Ornamental Pyrethroid Insecticide. The percentage of test article in the formulations studied ranged from 24.0-24.5%.

Consistent with the use patterns for this product, the following table summarizes the toxicity studies required to be submitted in support of the proposed registration (those requirements that have been satisfied are indicated):

	Cyfluthrin Technical		®Baythroid 240 Ornamental Pyrethroid Insecticide	
	Req.	Satis.	Req.	Satis.
Acute oral toxicity	yes	yes	yes	yes
Acute dermal toxicity	yes	yes	yes	yes
Acute inhalation toxicity	yes	yes	yes	yes
Primary dermal irritation	yes	yes	yes	yes
Primary eye irritation	yes	yes	yes	yes
Dermal sensitization	yes	yes		
21-day dermal	yes	yes		
21-day inhalation	yes	yes		
Teratology (2 species)	yes	yes		
Reproduction	yes	yes		
Chronic feeding (1 species)	yes	yes		
Oncogenicity (2 species)	yes	yes		
Metabolism	yes	yes		
Mutagenicity	yes	yes		
Special - "nervous system"	yes	Partially		

Due to concerns over the potential of cyfluthrin induced nervous system effects, the Toxicology Branch requested a hen brain neurotoxic esterase study (Doherty/Budd memorandum; 2-15-85; PP 4F3046). The Registrant submitted s NTE study, but it was classified as Core Supplementary because it was poorly reported and had study deficiencies. In addition, the Doherty/Budd memorandum requested the Registrant to resolve several questions regarding potential neurotoxicity in the hen study in question. The Registrant submitted comments on the hen study, but failed to address any of the questions posed to them.

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Two studies, a Subchronic Inhalation Study and a Mutagenicity Study were core classified as Invalid. These reports were lacking signatures of the performing scientists and Quality Assurance Officers. In a Doherty/Budd memorandum (PP 4F-3046/FAP 4H5427 and EPA Reg. No. 3125-GLR, 2-15-85), the failure of the Registrant to submit signed reports was cited along with the warning that studies would be classified Invalid unless signed reports were submitted. A memorandum from John Whalan (EPA No. 3125-GLR, May 8, 1986), reaffirmed this policy by stating that future studies would not be accepted without signatures. Accordingly, these reports will not be reviewed by the Toxicology Branch until they receive verifying signatures.

Another study, an Acute Oral Toxicity Study, was core classified as Invalid because of serious study deficiencies. None of the three invalid studies are required for registration of the technical or Baythroid 240 Ornamental Pyrethroid Insecticide.

Two other reports, comments by Drs. W. Flucke and J. Thyssen regarding the neurotoxicity of cyfluthrin, were also unsigned. They were reviewed because they were commentaries and did not present new data.

Dr. W. Flucke felt that the neurotoxicity observed in the hen studies was probably due to the vehicle, but in the absence of vehicle controls, there was no way to prove this hypothesis. Also, he demonstrated how cyfluthrin neurotoxicity differed from classical organophosphate delayed neurotoxicity. While there were definite differences in signs and chronology, it must be emphasized that pyrethroids are not organophosphates, and there is no reason to expect toxic manifestations to be similar.

Toxicology Branch cannot approve registration of this product for the following reasons:

1. Concerns regarding potential nervous system effects are not resolved. The Registrant must submit an acceptable neurotoxic esterase study and address the questions regarding potential neurotoxicity (Doherty/Budd memorandum; 2-15-85; PP 4F3046).

2. Concerns regarding human exposure have not been addressed. Toxicology Branch requested a human exposure assessment of the potential inhalation and dermal exposures that may result from the use of this product. (John Whalan memorandum; 3-12-85; EPA No. 3125-GLE). The Benefits and Use Division required the Registrant to provide the exposure information to permit EAB/HED to undertake their exposure assessment (William Phillips memorandum; 10-28-85; EPA No. 3125-GLE). A copy of this memorandum was given to the Registrant. As yet, no data has been supplied. Until these data are submitted, EAB cannot perform a human exposure assessment for dermal and inhalation exposure, and adequate margins of safety cannot be demonstrated between user exposure and the results of subchronic dermal and inhalation studies. Of particular concern are a 21-Day Rat Inhalation study with a NOEL of 0.0014 mg/l, and the Subchronic Inhalation study submitted in this action in which the summary describes an extremely low NOEL of 0.00009 mg/l (0.09 mg/m^3) [see attachment on page 22].

ACUTE INHALATION STUDY OF FCR 1272 IN RATS

Nihon Tokushu Noyaku Seizo K.K.; Report No. 269; February 3, 1984; Accession No. 261771

PROTOCOL: Six week old male (240-280 g) and female (165-190 g) Crj:CD rats were assigned to groups of 10/sex. They were dynamically exposed for 4 hours in a 42.4 liter inhalation chamber to nominal concentrations of 0 (air control), 0 (vehicle control), 2.731, 4.096, 4.779, and 6.144 mg/l. The test article (purity of 95.0%) was dissolved in a 1:1 mixture of ethanol and Lutrol (PEG 400), and generated as an aerosol by an unspecified method. All rats were observed daily for clinical signs, and weighed on days 0 (prior to exposure), 7 and 14. They were all necropsied and examined grossly. Chamber atmospheres were collected in three series connected Impinjors® and analyzed by gas chromotography. Food and water were available ad libitum.

RESULTS: The analytical chamber concentrations were as follows:

Chamber Concentrations (mg/l)	
Nominal	Analytical
2.731	0.339
4.096	0.955
4.779	1.085
6.144	1.124

There were no particle size data. There were no deaths in the control groups, or in the low dose group. The LC_{50} was calculated to be 1.010 (0.800-1.100) mg/l for males, and 1.020 (0.800-1.140) mg/l for females. Deaths in the three highest concentrations occurred between 2 hours of exposure and 2 hours after the end of exposure. Clinical signs included salivation, ataxia, lateral recumbency with flinging movements of the legs, piloerection, dyspnea, and straub tail. These signs reversed in the survivors within 5 days. Body weight gain was normal in all groups. The gross pathology results were uninterpretable.

This study is CORE INVALID. The test article was not characterized, and there were no analyses to assess formulation homogeneity. The gross pathology findings were poorly reported, making it impossible to determine which groups had lesions. There was an inadequate description of the inhalation chamber. A drawing in the report made it appear as though the rats were exposed nose-only, but there was no mention of this in the report. There was no mention of the method of generating an aerosol. There was no attempt to measure the particle size of the aerosol, thus making it impossible to assess the degree of respirable aerosol. Because of these deficiencies, the results of the study were uninterpretable. The study must be repeated. This study did not receive Quality Assurance review.

SUBCHRONIC INHALATION TOXICITY STUDY OF FCR 1272 IN RATS

Bayer AG Institute of Toxicology; Report No. 12436; February 1, 1984; Accession No. 261771

This study is INVALID. This report was lacking signatures of the performing scientists and Quality Assurance Officer. There was no summary of the histopathology data, and the individual histopathology tables were uninterpretable. There was scant description of the inhalation chamber and aerosol generator, and no mention of the placement of the rats in the chamber. [A copy of the summary from the report is attached on page 22.]

TERATOLOGY STUDY OF FCR 1272 IN RATS

Research & Consulting Company AG; Report No. 019348; December 14, 1983; Accession No. 261771

PROTOCOL: One hundred Wistar KFM-Han female rats (181-230 g, 12-13 weeks old) were assigned to four groups of 25 rats, and mated overnight with sexually mature males. Vaginal plugs and smears were used to establish gestation day 0. All mated dams were orally dosed with the test article (93.4% purity) once daily by intubation on days 6 through 15 at doses of 0 (vehicle control), 1, 3, and 10 mg/kg/day. The test article was formulated daily by mixing it with a vehicle of 1% Cremophor EL in distilled water. The test article was analyzed for homogeneity and dose concentration. The dams were observed twice daily for clinical signs, and weighed daily. Food consumption was measured on gestation days 6, 11, 16, and 21. The dams were asphyxiated, and their ovaries, uteri, uterine contents, and other organs examined. The fetuses were removed by caesarean section, sexed, weighed, and grossly examined. One third of the live fetuses from each litter were examined for visceral and brain lesions by the technique of Wilson (1965). The remaining live fetuses were clarified, stained with Alizarin red, and examined for skeletal lesions by the technique of Dawson (1926).

RESULTS: No dams died during this study. There were no clinical signs, except for vaginal bleeding (colporrhagia), abortion, and weight loss in one mid-dose dam on gestation day 18. Weight gain and food consumption were similar in all groups. The litter data were as follows:

Dose (mg/kg/d)	Pregnant/ Mated	Abortions	Live litters	Fetuses/dam	Fetuses % Live	Fetuses Wt (g)	Resorptions (%) Embryonic	Resorptions (%) Fetal
0	25/25	0	25	10.2	100	4.9	7.2	0
1	25/25	0	25	10.7	100	5.0	3.3	0
3	22/25	1	21	11.0	100	5.0	4.6	0
10	24/25	0	23	11.7	100	4.9	1.8	0

All groups had similar mean values for corpora lutea/dam, embryonic resorptions, implantations, live litters, fetuses/dam, male:female ratios, and fetal weights. There were no dead fetuses, and no fetal resorptions. There was however the one dam which aborted on day 18, but this was probably not a compound-related event.

Visceral lesions included coagulated blood in the abdominal cavity of one control and one high-dose fetus, and coagulated blood in the kidney pelvis of one low-dose and one high-dose fetus. Skeletal malformations and anomalies included absent sternebrae in 2 control, 3 low-dose, and 3 high-dose fetuses; longitudinally split sternebrae in 1 control, 1 low-dose, and 1 high-dose fetuses; and wavy ribs (all) in 1 control fetus. None of the visceral or skeletal findings are considered to be compound-related. Thus, there were no compound-related teratogenic effects at the doses tested. The defined NOEL for teratogenic, maternal toxicity, and fetotoxicity effects was $\geq$10 mg/kg/day in rats (the highest dose tested).

This study is CORE GUIDELINE. This study received Quality Assurance review.

SUBACUTE NEUROTOXICITY of Orally Administered FCR 1272 in Rats

Bayer AG Institut Fur Toxikologie; Bayer Report No. 12338; Mobay Report No. 86305; December 27, 1983

This study was previously reviewed (John Whalan memo, EPA No. 4F-3046, May 22, 1985), and classified Core Supplementary. The Registrant submitted an addendum to the report to resolve the report deficiencies. On the basis of the clarifications, the Core classification for this study was upgraded to Core Guideline (John Whalan memo, EPA No. 3125-GLR, May 17, 1986). In this current action, the report and addendum were resubmitted for an unspecified reason.

SUBACUTE NEUROTOXICITY of Orally Administered FCR 1272 in Rats

Nihon Tokushu Noyaku Seizo K. K. Agricultural Chemicals Institute; Mobay Report No. 86427; June 30, 1983

This study was previously reviewed (John Whalan memo, EPA No. 4F-3046, May 22, 1985), and classified Core Supplementary. The Registrant submitted a corrected report to resolve the report deficiencies. On the basis of the clarifications, the Core classification for this study was upgraded to Core Guideline (John Whalan memo, EPA No. 3125-GLR, May 17, 1986). In this current action, the Registrant resubmitted the corrected report for an unspecified reason.

NEUROTOXICITY STUDY OF THE EFFECT OF FCR 1272 ON NEUROTOXIC ESTERASE (NEUROTOXIC TARGET ENZYME) IN HENS

Bayer AG Institute of Toxicology; Report No. 13821; September 16, 1985; Accession No. 261433

This study was previously reviewed (John Whalan memo, EPA No. 3125-GLR, May 8, 1986), and classified Core Supplementary. The study was poorly reported and had many deficiencies. In this current action, the Registrant resubmitted an unsigned report for an unspecified reason.

THE EFFECT OF FCR 1272 ON NEUROMUSCULAR DYSFUNCTION IN RATS IN THE TILTING PLANE TEST

Toxikologisches Institut Regensburg; TIR Report No. 04003; Mobay Report No. 86726; May 4, 1984

PROTOCOL: The purpose of this study was to determine the degree of platform tilt at which a rat can prevent slipping when intoxicated with FCR 1272. It is a measure of corrective muscle contractions and feedback efficiency.

Groups of 10 fasted male Bor:WISW (SPF Cpb) rats (142-180 g; approximately 8 weeks old) were given oral acute doses (presumably by stomach tube) of the test article in 2% Cremophor EL/water, cypermethrin in 2% Cremophor EL/water, or Diazepam in 0.15% tragacanth mucilage/water by the following schedule:

	Test 1 (mg/kg)	Test 2 (mg/kg)	Test 3 (mg/kg)
Control	-	-	-
FCR 1272	0.1	0.01	
FCR 1272	0.3	0.03	
FCR 1272	1.0	0.1	
Cypermethrin			0.1
Cypermethrin			0.3
Cypermethrin			1.0
Diazepam	5.0	5.0	5.0

The rats were tested 0.5, 2, 5, and 7 hours after dosing. The platform was tilted until the rats began to slip. Each rat was tested 5 times on the tilted plane, and the mean of the measured angles was calculated and reported.

RESULTS: The mean slip angles for tests 1,2, and 3 were as follows (since lower doses were used in test 2, it is listed before test 1):

Compound	Dose (mg/kg)	Mean Slip Angle (°) at 0.5 hr.	2 hr.	5 hr.	7 hr.
TEST 2 -					
Control	-	36	36	36	36
FCR 1272	0.01	36	37	36	36
FCR 1272	0.03	36	36	36	34
FCR 1272	0.1	36	35	33	36
Diazepam	5.0	33	29	30	35
TEST 1 -					
Control	-	36	36	36	36
FCR 1272	0.1	36	35	32	34
FCR 1272	0.3	35	35	32	33
FCR 1272	1.0	35	34	29	32
Diazepam	5.0	33	29	30	33
TEST 3 -					
Control	-	40	40	39	38
Cypermethrin	0.1	40	41	39	39
Cypermethrin	0.3	40	39	37	39
Cypermethrin	1.0	40	37	33	38
Diazepam	5.0	38	36	36	39

In tests 1 and 2, the control rats had mean slip angles of 36° at each testing interval, and the diazepam treated rats had mean slip angles of 29-35°. In test 3, the mean slip angles increased significantly to 38-40° for the controls, and 36-39° for the diazepam treated rats. Because of this variability, it was impossible to compare values between tests. Compound-related effect was thus assessed within each test.

Diazepam treated rats had reduced mean slip angles at the 2 and 5 hour intervals with reversal by 7 hours. In rats treated with FCR-1272, there was no effect at the 0.01 mg/kg dose. Rats treated at 0.03 mg/kg had a 2° decrease at 7 hours compared to the controls; this was probably an anomaly. Rats treated at 0.1 mg/kg in test 2 had 2-3° decreases at 2 and 5 hours compared to the controls, but reversed by 7 hours. Rats also treated at 0.1 mg/kg in test 1 had 1-4° decreases at 2, 5, and 7 hours. More significant dose-related decreases were seen at doses of 0.3 and 1.0 mg/kg.

The lowest FCR 1272 dose at which there was a clear neurologic effect was 0.1 mg/kg. The 1.0 mg/kg FCR 1272 dose was equivalent to 5 mg/kg of diazepam. The lowest cypermethrin dose at which there was a clear neurologic effect was 0.3 mg/kg, at which there was a 1-2° decrease in the mean slip angles at 2 and 5 hours. Thus FCR 1272 is very slightly more toxic than cypermethrin as measured with the tilting plane test.

This study is ACCEPTABLE. The report failed to report whether the controls were dosed with a vehicle or left untreated. Individual animal data were not reported. This study did not receive Quality Assurance review.

COMMENTS ON REPORT NO. 9753 OF 27.1.1981 (FCR 1272 - NEUROTOXIC STUDY WITH CHICKENS BY DR. J. THYSSEN, DR. G. KALINER, AND DR. P. GROENING)

Bayer Institute of Toxicology; Report No. N/A; April 9, 1985 Accession No. 261433
Comments by: Dr. W. Flucke

BACKGROUND: This study was previously reviewed by John Doherty (PP 4F-3046/ FAP 4H-5427 and EPA Reg. No. 3125-GLR; 2-15-85). It was classified Core Supplementary because of a failure to use a proper solvent control group, and because there was no attempt to obtain a dose response. The study demonstrated that a dose of 5000 mg/kg/day given acutely, twice over a 3 week period, or 5 times over a week caused delayed neurotoxic signs and moderate fiber alterations in the sciatic nerve. These sciatic nerve alterations included axon fragmentation, occasional swelling and eosinophilia of the axon fragments, vacuolation and distension of the myelin sheaths, and optically void or granularly disintegrated myelin sheaths. There were also activated or proliferated Schwann's cells, and macrophages containing granular material.

AUTHOR'S COMMENTS: Based on the nature and onset of neurotoxic signs and lesions, Dr. Flucke questioned whether the findings were in fact delayed neurotoxic manifestations. He described classical delayed neurotoxicity as beginning with an uncertain gait, and progressing to frank ataxia, progressive severe ataxia, paresis, and paralysis; these signs occur between days 8 and 18. He discussed each portion of the study in turn. Toxicology Branch critiques and discussion on each point are in brackets "[]."

Acute Oral at a Dose of 5000 mg/kg:

1. Neurotoxic signs included disturbed behavior (day 14), prostration (day 15), and stiff limping gait (day 18). Dr. Flucke described the timing of these events as inconsistent with classical delayed neurotoxicity. [Classical delayed neuropathy is that caused by organophosphate poisoning. Pyrethroids are not known to cause delayed neurotoxicity. Cyfluthrin may be atypical for a pyrethroid in that it appears to cause a kind of delayed neuropathy; its nature need not be identical to the organophosphates.]

2. Despite the lack of controls for comparison, he feels that since the clinical signs in the two hens was different, yet the extent of sciatic trauma was the same, "a causal connection between clinical disorder and the observed nerve alterations does not seem plausible." [There is no assurance that the sciatic lesions were the cause of the clinical signs. Therefore, and particularly in the absence of controls, this disclaimer is unwarranted.]

3. Only 2 of 16 survivors had clinical evidence of neurotoxicity, and with different signs and times of occurrence. Dr. Flucke feels that this, "contradicts the presence of a causal connection of disorder and FCR 1272 treatment." [A true causal connection should have involved a greater percentage of hens.]

Double Oral Dose of 5000 mg/kg/day at an Interval of Three Weeks:

1. No delayed neurotoxicity was observed during the 3-week recovery period following the first dose. Five hens had neurotoxic signs after the second dose, including uncoordinated movements with collapse of the legs (day 13) followed by death, paralysis (day 15) followed by moribund sacrifice. "Complete paralysis does not develop so rapidly with delayed neurotoxicity, and mortalities normally do not occur just one day after the onset of the signs." [While this is certainly true of organophosphates, it may not be the case for pyrethroids.]

2. Another affected hen had disturbed behavior and uncoordinated leg movements (day 20) and was sacrificed one day later. Dr. Flucke described the timing of these events as inconsistent with classical delayed neurotoxicity. [What is true for organophosphates may not be true for pyrethroids.]

3. Another affected hen had disturbed behavior and uncoordinated leg movements (day 15) which persisted until it was sacrificed on day 21. It had lost a significant amount of weight. "These findings are also not typical of delayed neurotoxicity." [What is true for organophosphates may not be true for pyrethroids.]

4. Unlike the positive controls (TOCP), the histopathologic sciatic lesions in hens dosed with the test article did not correlate with the clinical signs, including paralysis. These lesions were likely "background neuropathologic changes which vary in degree and which are difficult to assess due to the absence of control findings." [There is no assurance that the sciatic lesions were the cause of the clinical signs.]

Five Oral Doses of 5000 mg/kg/day Over a Week:

1. Of the ten dosed hens, 6 survived five consecutive days of dosing. Three of these had disturbed behavior, sedation, and stiff gaits between days 25 and 32; they were sacrificed moribund. Dr. Flucke felt that the results were not typical of delayed neurotoxicity, that the signs and their times of occurrence were not consistent. "The signs and mortalities which occurred are therefore most likely to be attributable to disorders during the study, which weakened the animals and also resulted in their immobilization in moribund state (paralysis)." [What is true for organophosphates may not be true for pyrethroids.]

SUMMARY: According to Dr. Flucke, the neurotoxicity observed during this study deviated from that of the classical delayed neurotoxicity induced by organophosphates. Differences include the variations in symptoms, rapid onset of death, moribundity after losing weight, and the absence of dose-response with regard to clinical signs and histopathologic lesions. He cited another study in which hens dosed once or twice with 5000 mg/kg/day had no clinical signs of delayed neurotoxicity or nerve lesions. Cyfluthrin analogs including cypermethrin, deltamethrin, fenvalerate and permethrin do not cause neurologic signs or cause histopathologic nerve lesions. "There were therefore no indications that FCR 1272 could possess a neurotoxic potential for chickens."

[The test formulation did, of course, possess a neurotoxic potential for chickens as manifested by Dr. Flucke's descriptions of clinical signs and histopathologic evidence of nerve lesions observed in this study. In the absence of vehicle controls, it is impossible to ascertain whether the observed neurotoxicity was caused by the test article, the vehicle, or other unknown factors. No neurotoxicity was observed in three other studies in hens.]

[Dr. Flucke dismissed the observed neurotoxicity as not being typical delayed neurotoxicity since it did not resemble the signs and chronology of organophosphate poisoning. Since the pyrethroids are an entirely different class of pestides, there is no reason to assume that their toxic characteristics should resemble the organophosphates.

Unfortunately, Dr. Flucke failed to sign his report, and to address several issues itemized in John Doherty's review (PP 4F-3046/FAP 4H-5427 and EPA Reg. No. 3125-GLR; 2-15-85):

- Possible differences in the test material
  - ° Including a consideration of impurities, contaminants, and/or manufacturing by-products in the test material.
  - ° Including a consideration of possibly different ratios of active ingredient isomers in the test material.
- Possible differences in the test animals used
  - ° Including a consideration of strain, source, etc.

° Including a consideration of normal background incidence of nervous system lesions in historical control animals of the same strain and source (if possible).

- Possible differences in investigational techniques employed

These questions remain unanswered.

COMMENTS ON THE EFFECT OF CYFLUTHRIN ON NERVE TISSUE WITH SPECIAL REFERENCE TO THE HEALTH HAZARD FOR USER AND CONSUMER

Bayer Institute of Toxicology; Report No. N/A; February 22, 1983; Accession No. 261771

AUTHOR'S COMMENTS: Dr. J. Thyssen presented an overview of the neurotoxic effects of pyrethroids based on in vivo and in vitro findings reported in the literature. He then commented cyfluthrin with regard to the neurotoxic findings and its potential health hazards.

Pyrethroids induce membrane alterations in nervous tissue which alter the flow of sodium and potassium and the action potential, resulting in depolarization and loss of synaptic transmission. Those containing CN are particularly effective. Pyrethroids inhibit the activity of adenosine triphosphatase (ATP-ase) which controls the sodium/potassium pump. They also affect the neurotransmitters cyclic guanosine monophosphate (cGMP) and gamma aminobutyric acid (GABA).

The administration of cypermethrin to rats and hamsters at potentially lethal doses resulted in axonal degeneration, as manifest by increased beta glucuronidase activity. The same nerve lesions were seen in rats dosed with potentially lethal doses of fenvalerate, resmethrin, permethrin, deltamethrin, and natural pyrethrum. Cyfluthrin does not inhibit ATPase, but 5 of its metabolites do.

There were no deaths and no nerve lesions in hens dosed dermally with 5000 mg/kg/day on 5 x 23 hour and 15 x 6 hour regimens, or in hens dosed by inhalation to an aerosol concentration of 0.614 mg/l on a 15 x 6 hour regimen. Similarly, there were no nerve lesions in rabbits dosed dermally at a maximum dose of 250 mg/kg/day on a 15 x 6 hour regimen, or in rats dosed by inhalation to an aerosol concentration of 0.070 mg/l on a 15 x 6 hour regimen.

Rats dosed in their feed at 300 ppm (15 mg/kg/day) for 3 months, and dogs dosed in their feed at 600 ppm (15 mg/kg/day) also had no nerve lesions. Rats orally dosed at 60-80 mg/kg/day for 5 months had "typical signs of pyrethroid poisoning, and a small number of animals died," but there was no paralysis or nerve damage. The most sensitive species was reported to be the dog which had an oral NOEL of 1.63 mg/kg/day (65 ppm) in a 6-Month Feeding Study.

Dr. Thyssen calculated human NOEL values. His methods and reasons for selecting uncertainty factors was not clear, and may have been in error. There was at least one discrepancy (in the extrapolated dermal dose), and not all routes were considered. Therefore, these calculations are not presented here. As of the date of his report, Dr. Thyssen claimed that there have been no cases

of nerve damage in users of any pyrethroid products anywhere in the world. "As the nerve damage observed only occurred in the lethal dose range, and since these lethal doses are impossible for consumers eating foodstuffs containing cyfluthrin, and also on the strength of the extremely high safety factors calculated, a health hazard in respect of nerve damage may be ruled out for consumers. A health hazard in respect to nerve damage may not therefore be anticipated, either when formulations containing cyfluthrin are used, or when foodstuffs containing cyfluthrin are eaten."

FCR 1272 INDUCED SISTER CHROMATID EXCHANGE ASSAY IN CHINESE HAMSTER OVARY (CHO) CELLS

Microbiological Associates, Inc.; Report No. 693; September 30, 1985; Accession No. 261771

PROTOCOL: This study was performed in two parts. The doses selected for use in the SCE assay were based on the results of a cytotoxicity test. In the cytotoxicity test, Chinese hamster ovary cells were seeded in flasks and incubated for 16-24 hours. They were treated with 5 ml of complete medium (nonactivated systems) or 5 ml of the S-9 mixture (activated systems) which contained 50 ul of the test article (technical - 94.7% pure) in acetone at concentrations of 0.1-1000 ug/ml, or the vehicle (acetone). The test article precipitated at the 1000 ug/ml concentration. Each dose level was tested in duplicate. The nonactivated cells were incubated for 20-24 hours. The activated cells were incubated for 2 hours, then rinsed with phosphate buffered saline (PBS), refed with 5 ml of complete medium, and further incubated for a 20-24 hour recovery period. The cells were harvested and counted. The S-9 mixture was prepared from hepatic microsomes (S-9) of Arochlor 1254 dosed male Sprague-Dawley rats, plus a co-factor generating system (isocitric acid and NADP).

In the SCE assay, CHO cells were seeded, incubated, and dosed as in the cytotoxicity test. The test article concentrations were 3, 5, 10, and 20 ug/ml for the nonactivated systems, and 125, 250, 500, and 1000 ug/ml in the activated systems. There were also untreated and vehicle controls. The positive controls were dosed with 0.025 ug/ml of triethylenemelamine (TEM) for the nonactivated systems, and 2.5 ug/ml of cyclophosphamide (CP) for the activated systems. Each dose level was tested in duplicate.

The CHO cells in the nonactivated systems were dosed for periods of 26-32 hours. After the second hour, 0.01 mM of 5-bromo-deoxyuridine (BrdUrd) was added to the medium. Colcemid (0.1 ug/ml) was added to the medium during the final 2-3 hours of incubation.

The CHO cells in the activated systems were dosed for periods of 2-3 hours. They were then washed with PBS, refed with complete medium containing 0.01 mM of 5-bromo-deoxyuridine (BrdUrd), and further incubated for 24-30 hours. Colcemid (0.1 ug/ml) was added to the medium during the final 2-3 hours of incubation.

Metaphase cells were harvested by mitotic shake-off, fixed with Carnoy's fixative, and stained by a modified fluorescence-plus-Giemsa technique. Fifty

cells per treatment level were evaluated for the percentage of cells in the first (M_1), second (M_2), or third (M_3) metaphase division.

RESULTS: In the cytotoxicity test, dose-related decreases in survivability were observed at concentrations >3 ug/ml in the nonactivated systems, and >100 ug/ml in the activated systems, compared to the vehicle controls. In the SCE Assay, the untreated and vehicle controls in the nonactivated and activated systems were similar, whereas the positive controls in each system had 4-fold increases in SCE's per chromosome and mean SCE's per cell. There were no increases in SCE frequency in cells treated with the test article at any dose, however. The only effect seen in cells treated with the test article was a significant increase in cells failing to progress to the M_2 metaphase division at the 20 ug/ml concentration in the nonactivated system; this was was a result of cytotoxicity at this dose, but was not seen in the activated systems. Thus, the test article was not mutagenic - even at doses which were cytotoxic (nonactivated systems) or at the limit of solubility (activated systems).

This study is ACCEPTABLE. This study received Quality Assurance review.

FCR 1272-INDUCED CHO/HGPRT MUTATION IN THE PRESENCE AND ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Microbiological Associates, Inc.; Report No. 694; September 30, 1985; Accession No. 261771

PROTOCOL: This study was performed in two parts. The doses selected for use in the mutation assay were based on the results of a preliminary toxicity test. In the preliminary toxicity test, Chinese hamster ovary cells were seeded in flasks and treated with DMSO (vehicle) and test article (94.7% purity) concentrations which ranged from 0.001-10 ul/ml, both in the presence and absence of S-9 mixture. The S-9 mixture was prepared from hepatic microsomes (S-9) of Arochlor 1254 dosed male Sprague-Dawley rats, plus a co-factor generating system (isocitric acid and NADP). The doses selected for use in the mutation assay were based on cloning efficiency relative to the vehicle controls.

In the mutation assay, growing CHO-K_1-BH_4 cells were plated in flasks at a density of 5×10^5 cells/flask, and incubated for 18-24 hours. These cells were treated in duplicate with medium containing 3, 5, 7, 9, and 10 ul/ml test article concentrations (technical - 94.7% pure) in DMSO, and incubated for 5 hours. The activated systems were treated with medium which contained S-9 mixture. Cytotoxicity (cloning efficiency) of these colonies was assessed. After the incubation period, the media was aspirated, the cells were washed with calcium and magnesium free HBSS, and incubated in Ham's F-12 medium (F12FBS5) for an additional 18-24 hours. The cells were then subcultured to evaluate cytotoxicity and allow for phenotypic expression.

Cytotoxicity was evaluated by subculturing replicates in F12FBS5 in triplicate, and culturing the cells for 7-10 days. The colonies were fixed with methanol, stained with 10% aqueous Giemsa, and counted.

The expression of mutant phenotypes was revealed by subculturing replicates in F12FBS5 in duplicate, and culturing the cells for a 7-9 day expression period. The replicates were replated in quintuplicate in petri dishes in F12FBS5-Hx containing 10uM 6-thioguanine. Cloning efficiency was assessed by replating 100 cells/dish in triplicate, and incubating for 7-10 days. The colonies were fixed, stained, and counted for mutant selection and cloning efficiency.

Ethyl methanesulfonate (EMS) was used as the positive control article in the nonactivated systems, and benzo(a)pyrene (BaP) was used as the positive control article in the activated systems. The vehicle control article was DMSO.

RESULTS: In the preliminary toxicity test, cloning efficiency was reduced moderately at 10 ul/ml in the nonactivated and activated system. In the mutation assay, there was no significant cytotoxicity (decreased cloning efficiency) in the nonactivated systems treated with test article concentrations ranging from 3-10 ul/ml for 5 hours. The EMS treated positive controls had cloning efficiency which was one-third that of the solvent controls. In the mutation assay, the incidence of total mutant colonies in the cultures treated with the test article was similar to that of the solvent controls. The mutation frequency (mutants/10^6 clonable cells) was increased 4-fold in the 10 ul/ml dose, and 76-fold in the EMS positive controls, relative to the solvent controls.

Because of the lack of clear dose-related effect, the cytotoxicity and mutation assays were repeated for the nonactivated systems. Once again, there was no significant cytotoxicity (decreased cloning efficiency) in the nonactivated systems treated with test article concentrations ranging from 3-10 ul/ml for 5 hours. The EMS treated positive controls had cloning efficiency which was one-fourth that of the solvent controls. In the mutation assay, the incidence of total mutant colonies in the cultures treated with the test article was less than that of the solvent controls. The mutation frequency was also less in the colonies treated with the test article, but there was a 27-fold in the EMS positive controls, relative to the solvent controls.

Similarly, there was no significant cytotoxicity (decreased cloning efficiency) in the activated systems in the cells treated with test article concentrations ranging from 3-10 ul/ml for 5 hours. The BaP treated positive controls had cloning efficiency which was one-half that of the solvent controls. In the mutation assay, the incidence of total mutant colonies in the cultures treated with the test article was similar to that of the solvent controls. The BaP treated positive controls had total mutant colonies which were 19-fold that of the untreated controls (the solvent controls had no mutant colonies). The mutation frequency for the cells treated with the test article was lower than for the untreated controls. The solvent controls had lower mutation frequency than the untreated controls. The BaP positive controls had a mutation frequency 30-fold that of the untreated controls.

Thus, the positive controls clearly elicited thioguanine-resistant mutants, but the test article was not mutagenic.

This study is ACCEPTABLE. There was no explanation for the differences in the two nonactivated assays. This study received Quality Assurance review.

FCR 1272-INDUCED UNSCHEDULED DNA SYNTHESIS IN RAT PRIMARY HEPATOCYTES

Microbiological Associates, Inc.; Report No. 701; December 30, 1985; Accession No. 261771

PROTOCOL: The hepatocytes used in this study were obtained from an unspecified number of healthy adult male Sprague-Dawley rats according to the procedure of Williams, et al (1977). This study was performed in two parts. In the cytotoxicity test, replicate hepatocyte cultures were treated 90-120 minutes after seeding with concentrations of the test article ranging from 0.07-2000 ug/ml. The test article (94.7% pure) was dissolved in an acetone/water vehicle. Toxicity was tested 18-20 hours later by washing the cultures with calcium and magnesium free phosphate buffered saline, trypsinization, and staining with trypan blue. This test was done in duplicate. The results of this test were used to select the doses in the UDS test.

In the unscheduled DNA synthesis test, sets of three replicate plates were dosed with 17, 50, 167, 500, 1667, and 5000 ug/ml of the test article in acetone/water, with 3 and 10 ug/ml 7,12-dimethylbenzanthracene (DMBA) in DMSO/water as the positive control, with 10 ul/ml acetone in water (vehicle control), with 3 and 10 ul/ml DMSO in water (vehicle controls), and with Williams Medium E (untreated controls). Each petri dish was further dosed with 10 uCi/ml of ^{3}H-thymidine. A duplicate set of plates were obtained for use in a toxicity test. All cultures were treated for a period of 18-20 hours. The plates set aside for the toxicity test were harvested by trypsinization, processed as above, and evaluated for toxicity. The plates used in the UDS test were washed in serum-free WME, fixed, mounted on slides, dried, and coated with Kodak NTB emulsion. After 10 days of dark desiccated storage (4°C), the slides were developed with Kodak D-19, fixed, and stained with hemotoxylin-sodium acetate-eosin. UDS was scored by counting nuclear grains in 25 randomly selected cells/slide (3 slides per treatment level). Nuclei which were damaged, small, or undergoing replication (evidenced by completely blackened nuclei) were excluded from the counts.

RESULTS: In the cytotoxicity test, cell viability at doses ranging from 0.07 to 2000 ug/ml was similar to that for the acetone vehicle. Accordingly, the doses selected for the UDS test covered a range of 17-5000 ug/ml. In the unscheduled DNA synthesis test, the survival index in the toxicity test was 38.0% for the acetone controls, 39.2% for the DMSO controls, and 34.4% for the untreated controls. Test article values ranged from a nadir of 17.2% at 500 ug/ml to a zenith of 36.4% at 5000 ug/ml, the highest dose. The increased survival at the highest dose was attributed to precipitation, which may have resulted in a significantly weaker solution. The survival index for cultures dosed with DMBA (positive control) was 17.2% at 3 ug/ml, and 9.4% at 10 ug/ml. These data demonstrate the cytotoxicity of the positive control article (DMBA), and also indicate that the test article was cytotoxic at doses of 17-1667 ug/ml.

In the UDS test portion of the study, the mean count of nuclear grains/nucleus was 0.7 for the acetone controls, 0.4 for the DMSO controls, and 0.5 for the untreated controls. The mean counts for the cultures treated with the test article ranged from 0.0 to 0.4, similar to the controls. None of the test or control cells had >5 grains/nucleus. The cells treated with DMBA, however, all had >5 grains/nucleus, and mean counts of nuclear grains/nucleus of 25.3 at 3 ug/ml and 27.2% at 10 ug/ml. This demonstrated that DMBA was a potent

inducer of unscheduled DNA synthesis, whereas the test article was not (up to levels of cytotoxicity).

This study is ACCEPTABLE. This study received Quality Assurance review.

SALMONELLA/MICROSOME TEST TO EVALUATE FOR FPBA-INDUCED POTENTIAL POINT MUTATION

Bayer AG Institute of Toxicology; Report No. 13429; April 22, 1985; Accession No. 261771

This study is INVALID. This report was not reviewed because it was lacking signatures of the performing scientists and Quality Assurance Officer. [redacted]

INERT INGREDIENT INFORMATION IS NOT INCLUDED

PRODUCT IMPURITY INFO IS NOT INCLUDED

ACUTE ORAL TOXICITY OF ®BAYTHROID C 2 EC IN RATS

Mobay Chemical Corporation; Report No. 695; November 21, 1985; Accession No. 261771

PROTOCOL: Male (302-369 g) and female (210-261 g) Sprague Dawley rats were randomly assigned to groups of 5 rats/sex/group. Fasted animals were acutely dosed by gavage with 500, 1000, and 1500 mg/kg of the test article (24.3% a.i.) in deionized water (dose volume of 1 ml/100 g). All rats were observed daily for clinical signs, and weighed on days 0 (prior to treatment), 7, 14, and terminally. They were all necropsied and examined grossly. Food and water were available ad libitum.

RESULTS: There were deaths in all dose levels, with the highest dose being lethal to all rats in the group. The days of death were not specified. The LD_{50} values were calculated to be 647 (366-1086) mg/kg for males, and 695 (497-965) mg/kg for females. Clinical signs included writhing, decreased activity, diarrhea, urine stained fur, and salivation. Additionally, tremors, ataxia, lacrimation, and piloerection were seen in the mid and high-dose groups. All clinical signs reversed by day 5. There was a significant reduction in the body weight gain of the mid-dose rats relative to the low-dose group (there were no controls against which to compare body weights). Among the rats which died during the study, dark red lungs were found in all male groups and the high-dose females.

This study is CORE MINIMUM, Toxicity Category III. There was no mention of the rat's ages. This study received Quality Assurance review. The Quality Assurance Manager, R.S. Schroeder released the report although it was in GLP violation, since, "The mixture of the test substance with the carrier was not analyzed for homogeneity, stability, or concentration of the test substance."

ACUTE DERMAL TOXICITY OF ®BAYTHROID C 2 EC IN RABBITS

Mobay Chemical Corporation; Report No. 665; September 24, 1985; Accession No. 261771

PROTOCOL: Groups of 5 male (2.20-2.59 kg) and 5 female (2.27-2.67g) adult New Zealand White rabbits were dermally dosed with 2000 mg/kg of the test article (24.3% a.i.) on the shaved skin of their backs (240 cm^2 area). The dosing sites were covered with gauze secured with hypoallergenic tape, then occluded with plastic film, tape, and an elastic bandage. Each rabbit was also fitted with a plastic collar. After 24 hours of exposure, the dosing sites were wiped clean with a paper towel moistened with water. The rabbits were observed daily for clinical signs, and weighed on days 0 (pretreatment), 7, 14, and terminally. All rabbits were necropsied and examined grossly. Food and water were available ad libitum.

RESULTS: There were no deaths in this study, so the LD_{50} was >2000 mg/kg. The only clinical sign observed was dosing site erythema which reversed by day 6 in females, and by day 13 in males. The rabbits failed to gain weight during the first week, but the significance of this finding cannot be determined without controls. There were no compound-related gross lesions.

This study is CORE MINIMUM, Toxicity Category III. There was no mention of analyses to characterize the test article, or assess formulation homogeneity and dose concentration. This study received Quality Assurance review.

ACUTE INHALATION STUDY OF BAYTHROID C 2 EC IN RATS

Mobay Chemical Corporation; Report No. 697; December 10, 1985; Accession No. 261771

PROTOCOL: Groups of 10 male (197-284 g) and 10 female (175-218 g) Sprague-Dawley rats were dynamically exposed (head only) in a 60 liter cylindrical chamber for 4 hours at nominal concentrations ranging from 1.180-5.200 mg/l. Two air control groups were treated as well. The test article (24.5% a.i.) was generated as an aerosol by a nebulizer. Particle size distribution near the rats' breathing zone was measured with a Mercer® Cascade Impactor. Analytical chamber concentration was assessed by collecting aerosol near the rats' breathing zone onto Millipore® FH 0.5um filters, then analyzing the deposits on a HPLC. All rats were observed several times during and after exposure, then twice daily for clinical signs, and weighed on days 0 (prior to exposure), 3, 7 and 14. They were all necropsied and examined grossly. Food and water were available ad libitum.

RESULTS: The analytical chamber concentrations were as follows:

Chamber Concentrations (mg/l)		
Nominal		Analytical
1.180		0.438
1.370		0.584
1.648		0.604
2.060		0.901
3.252		1.277
3.900	[females only]	1.565
5.200	[1 hr. exposure]	2.029

The particle size data were not broken out by chamber concentrations, but most of the values were similar. The mean of 13 atmosphere measurements was a mass median aerodynamic diameter of 2.1 um with a geometric standard deviation of 2.5. This suggests that a substantial percentage of particles were respirable.

There were no deaths in the control groups, or in the low dose group. There were also no deaths in the 2.029 mg/l groups which were dosed for only 1 hour. Death in the lethal concentrations occurred within 1 day of exposure. The LC_{50} was calculated to be 0.716 (0.613-0.851) mg/l for males, and 0.924 (0.781-1.118) mg/l for females (probit analyses excluded the 2.029 mg/l groups which were dosed for only 1 hour, and the 0.584 mg/l male which was suffocated by the latex collar damming).

Clinical signs in rats exposed for 4 hours included convulsions, salivation, ataxia, decreased activity, bloody oral discharge, and ocular and nasal irritation. The rats dosed at 2.029 mg/l for 1 hour had salivation, increased

and decreased activity, and ataxia. There were significant decreases in weight gain on days 3 and 7 in the males exposed for 4 hours in the 1.277 mg/l chamber, and in the females exposed for 4 hours in the 1.565 mg/l chamber. These decreases had reversed by day 14. Gross lesions seen in rats found dead included red turbinates, red cervical lymph nodes, salivation, red oral discharge, tan nasal discharge, mottled red thymus, red salivary gland, white eyes, red jejunal wall and ingesta, and neck edema. The latex collars were probably the cause of the neck edema, reddened cervical lymph nodes, and mottled red thymus. There were no compound-related gross lesions seen in rats sacrificed at the end of the study. There were no significant gross lesions in rats exposed to 2.029 mg/l for 1 hour.

This study is CORE MINIMUM, Toxicity Category III. Particle size distribution data were reported, but it was impossible to match particle sizes with chamber concentrations. This study received Quality Assurance review. The Quality Assurance Manager, R.S. Schroeder released the report although it was in GLP violation, since, "The mixture of the test substance with the carrier was not analyzed for homogeneity or stability of the test substance."

PRIMARY SKIN IRRITATION STUDY OF BAYTHROID C 2 EC IN RABBITS

Mobay Chemical Corporation; Report No. 675; October 8, 1985; Accession No. 261771

PROTOCOL: Six adult New Zealand White rabbits (sex, weight, and age unknown) were dermally dosed with 0.5 ml aliquots of the undiluted test article (24.3% a.i.) on the shaved skin of their backs and sides (6 cm^2 area). The dosing sites were covered with gauze and occluded with plastic film and an elastic bandage. After 4 hours of exposure, the dosing sites were wiped clean with a paper towel moistened with water. The dosing sites were scored for irritation 30 and 60 minutes, 24, 48, and 72 hours after dose removal. If irritation was present at 72 hours, the dosing sites were evaluated again at 7 days, and weekly thereafter until the lesions had reversed. Food and water were available ad libitum.

RESULTS: Dosing site erythema was seen in all rabbits (between 1 hour and 14 days) and ranged from very slight to well-defined. Most lesions reversed by day 7. Only 1 rabbit had very slight erythema at 14 days, and this lesion reversed by 21 days. Edema was seen in 3 of 6 rabbits (between 24 and 72 hours) and ranged from very slight to slight. The Study Report attributed this irritation to the [redacted] vehicles rather than to the test article. This assumption cannot be addressed since there were no vehicle controls.

This study is CORE MINIMUM, Toxicity Category III. The test article was not characterized. This study received Quality Assurance review.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

PRIMARY EYE IRRITATION STUDY OF BAYTHROID 240 EC FORMULATION C IN RABBITS

Mobay Chemical Corporation; Report No. 449; December 1, 1983; Accession No. 261771

PROTOCOL: Six adult New Zealand White rabbits (sex, weight, and age unknown) were treated by instilling 0.1 ml aliquots of the undiluted test article (24% a.i.) into their left eyes. The right eyes served as controls. Their eyes were not washed. Two to three minutes prior to dosing, the treated eyes were anesthetized with proparacaine. The treated eyes were evaluated for irritation 1, 24, 48, and 72 hours after dosing according to the method of Draize (1956). If irritation was present at 72 hours, then examinations continued as needed on days 7, 8, 14, and 21. Food and water were available ad libitum.

RESULTS: Corneal opacity was observed between 1 hour and 21 days, and ranged from diffuse to easily discernible. Marked iridic lesions occurred between 1 hour and 8 days. Conjunctival redness ranged from mild injection to diffuse beefy red, and occurred between 1 hour and 8 days. Chemosis ranged from mild to swelling with lids nearly or completely closed, and occurred between 1 hour and 21 days (two rabbits still had chemosis at 21 days). Discharge ranged from mild to marked, and occurred between 1 hour and 14 days. These lesions were seen in all rabbits, and were generally most severe during the first day.

This study is CORE MINIMUM, Toxicity Category II. The data tables were barely readable. The test article was not characterized. This study received Quality Assurance review.

DERMAL SENSITIZATION (BUEHLER TOPICAL CLOSED-PATCH TECHNIQUE) STUDY OF BAYTHROID C 2 EC IN MALE GUINEA PIGS

Mobay Chemical Corporation; Report No. 689; November 21, 1985; Accession No. 261771

PROTOCOL: Twenty-seven adult male Hartley guinea pigs (weight, and age unknown) were selected for this study. Fifteen guinea pigs were selected to receive induction treatments on days 0, 7, and 14. They were dosed with 0.5 ml aliquots on their shaved left flanks. Undiluted test article (24.3% a.i.) was applied on days 0 and 7, and a 50% dilution was used on day 14. The dosing sites were covered with two layers of gauze and occluded with plastic film and an elastic bandage. After 6 hours of exposure, the coverings were removed (presumably, the induction doses were not removed). The inducted skin sites were scored for irritation at 24 and 48 hours after dose application, and on days 8, 9, 15, and 16.

The 15 inducted guinea pigs and another 5 untreated guinea pigs were challenged on day 28 by applying 0.5 ml of a 50% aqueous test article dilution to their shaved left flanks, and 0.5 ml of a 50% aqueous vehicle dilution (Baythroid C 2 EC, without cyfluthrin) to their shaved right flanks. The challenge doses were occluded for 24 hours. The vehicle was sufficiently irritating to preclude meaningful interpretation of the test article's sensitizing potential. Thus, the guinea pigs were similarly challenged 2 weeks later (day 42), but this time with 12.5% aqueous dilutions of the test article and vehicle.

The dosing sites were scored for irritation 24 and 48 hours after each induction dose application, and 48 and 72 hours after each challenge dose application. Food and water were available ad libitum.

RESULTS: The induction doses caused erythema which worsened with each of the three applications. The erythema ranged from none to well-defined, and involved all treated guinea pigs. As expected, the challenge doses (50% dilutions) caused erythema which ranged from none to well-defined. Erythema of the right flank (treated with the vehicle) was more severe than that of the left flank (treated with the test article). The control animals, which had not received induction doses, had similar reactions. Thus, any sensitization potential was obscured by the severity of the vehicle-induced irritation.

When the guinea pigs were again challenged two weeks later with weaker (12.5%) dilutions of the test article and vehicle, the inducted animals had erythema which ranged from none to well-defined 48 hours after challenge dose application, and from none to very slight 72 hours after challenge dose application. Once again, the right flank (treated with the vehicle) was more severely affected than the left flank (treated with the test article). The control animals, which had not received induction doses (different animals from those used as controls in the first challenge), had no irritation at all when treated with the test article and vehicle.

DISCUSSION: The control animals used in the second challenge had not been treated at all until the time of the challenge. When they were treated with the 12.5% dilutions of the test article and the vehicle, they did not respond with any erythema. The test article formulations were less irritating that the vehicle formulations, presumably because the irritants in the vehicle formulations were in greater concentration than in the test article formulations. Thus, Baythroid C 2 EC is a skin sensitizer in guinea pigs, probably as a result of one or more of the vehicle components.

This study is CORE MINIMUM. This study received Quality Assurance review. The Quality Assurance Manager, R.S. Schroeder released the report although it was in GLP violation, since, "The mixture of the test substance with the carrier was not analyzed for homogeneity, stability, or concentration of the test substance."
