# TOPICAL DISCUSSIONS

## ACEPHATE

(O,S-Dimethyl acetylphosphoramidothioate)

Prepared by Krystyna K. Locke, Toxicologist

The mutagenicity section was prepared by
William R. Schneider, Microbiologist

TABLE OF CONTENTS

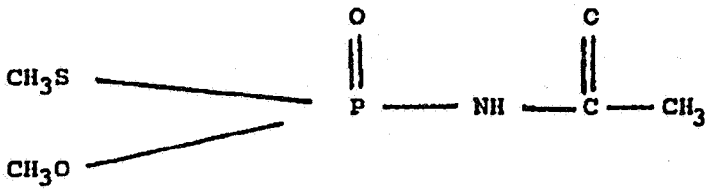
003122


| | | Page |
|---|---|---|
| | ACUTE TESTING | |
| 163.81-1 | Acute oral toxicity | 2 |
| 163.81-2 | Acute dermal toxicity | 3 |
| 163.81-3 | Acute inhalation toxicity | 4 |
| 163.81-4 | Primary eye irritation | 6 |
| 163.81-5 | Primary dermal irritation | 7 |
| 163.81-6 | Dermal sensitization | 8 |
| 163.81-7 | Acute delayed neurotoxicity | 10 |
| | Special acute studies | |
| | Intraperitoneal toxicity | 11 |
| | Potentiation of acute oral toxicity | 11 |
| | Cholinesterase inhibition | 15 |
| | SUBCHRONIC TESTING | |
| 163.82-1 | Subchronic oral toxicity | 17 |
| | Subchronic oral toxicity: cholinesterase inhibition | 18 |
| 163.82-2 | Subchronic 21-day dermal toxicity | 25 |
| 163.82-3 | Subchronic 90-day dermal toxicity | 25 |
| 163.82-4 | Subchronic inhalation toxicity | 26 |
| 163.82-5 | Subchronic neurotoxicity | 26 |
| | CHRONIC TESTING | |
| 163.83-1 | Chronic feeding | 27 |
| 163.83-2 | Oncogenicity | 32 |
| 163.83-3 | Teratogenicity | 36 |
| 163.83-4 | Reproduction | 38 |
| | MUTAGENICITY TESTING | |
| 163.84-1 through 4 | | 39 |
| | METABOLISM | |
| 163.85-1 | | 41 |
| | AUGMENTATION TOPICS | |
| | In vitro studies of acetylcholinesterase | 46 |

ACEPHATE, ORTHENE, ORTHO 12420, RE 12420

ORTRAN, ENT-27822

003122




O,S-Dimethyl acetylphosphoramidothiate

Acephate is a common name for O,S-dimethyl acetylphosphoramidothioate. Acephate is an analogue and a derivative of methamidophos (O,S-dimethyl phosphosamidothioate; Monitor). It possesses high insecticidal activity and, unlike methamidophos, very low toxicity for warmblooded animals. The French company, Chevron Chemical Company, began marketing it in 1974 under the trade name Orthene and the code designations RE 12420 and Ortho 12420. In the United States, the names Acephate, Orthene, Ortho 12420 and RE 12420 are used synonymously.

Acute Oral Toxicity (163.81-1)

003122

The minimum data requirement for testing acute oral toxicity ($LD_{50}$) is one test on the technical chemical and on each manufacturing use and formulated product, preferably using the laboratory rat.

Orthene Technical (ORTHO 12420, RE 12420) was slightly toxic (Category III) to male and female Sprague-Dawley rats after the animals were exposed to single doses ranging from 400 to 2020 mg/kg of body weight (Cavalli, 1970, MRID 00014675. The toxic signs observed at all exposure levels were those generally associated with the inhibition of cholinestrase activity, such as tremors, salivation, rhinorrhea, depression and labored breathing. In most instances, deaths occurred after 48 hours following exposure. Animals which died before the termination of the study showed pulmonary edema and pulmonary congestion. Survivors autopsied 14 days after the treatment had no gross abnormalities in all of the organs or tissues examined (lungs, heart, thymus, liver, kidney, spleen, gastro-intestinal tract, adrenal glands, gonads, pancreas, bladder, body fat, skeletal muscle and skin). In this study, the $LD_{50}$ values for male and females rats wre 945 and 866 mg/kg, respectively. This study is adequate.

Toxic symptoms usually associated with the inhibition of cholinesterase activity, as well as decreased food consumption, ataxia, bloody tears and diarrhea, were also observed in another study with male and female rats and Orthene 97% Technical (known also as Orthene Specialty Concentrate) as the test substance (Rittenhouse, 1977, MRID 00029696). In this study, Sprague-Dawley rats received single doses of the test material ranging from 500 to

2500 mg/kg. In the surviving rats, all toxic symptoms disappeared in 2-7 days after the exposure. No gross pathological changes were observed in twelve organs or tissues examined. The $LD_{50}$ values were 1000 mg/kg for female rats and 1400 mg/kg for male rats. Brain tissue was not examined in neither of these two studies. This study is adequate.

The above information is sufficient to satisfy the data requirement.

Acute Dermal Toxicity (163.81-2)

The minimum data requirement for testing acute dermal toxicity ($LD_{50}$) is one test on the technical chemical and on each manufacturing use and formulated product, preferably using the albino rabbit.

Orthene Technical had low toxicity (Category III) by the dermal route. At the exposure level of 2 g/kg for 24 hours, there were no toxic signs, deaths, or gross tissue abnormalities in the four male New Zealand rabbits, two with intact and two with abraded skin, studied by Cavalli (1970, MRID 00014681). These data are of supplementary nature.

In another study, male New Zealand rabbits, three with intact and three with abraded skin per level, were exposed to 5 or 10g of Orthene Technical/kg for 24 hours (Chevron Chemical Company, 1977, MRID GS0042-007). The following results were obtained:

1. There were no deaths.

2. Tremors were observed in most of the rabbits shortly after dosing, but the animals were normal the following day.

3. Diarrhea was observed in five rabbits at the 10 g/kg level, but disappeared during the 14-day observation period.

4. There were no significant differences in the weight gain between the control and the treated groups.

5. No gross pathological changes were observed in the eighteen organs or tissues examined that could be attributed to the test material.

Based on this study, the acute dermal $LD_{50}$ value for male rabbits is greater than 10 g/kg.

This study is adequate. Although only male rabbits were used, there were no deaths at the very high exposure levels and, therefore, additional tests with female rabbits appear unnecessary.

The above information is sufficient to satisfy the data requirement.

Acute Inhalation Toxicity (163.81-3)

The minimum data requirement for testing acute inhalation toxicity ($LC_{50}$) is one test on the technical chemical and on each manufacturing use and formulated product, preferably using the laboratory rat.

An acute inhalation toxicity ($LC_{50}$) test is required for each formulation that causes a respirable vapor, or if 20% or more of the aerodynamic equivalent is composed of particles not larger than 10 microns.

Orthene 97% Technical (Orthene Specialty Concentrate) was not toxic (Toxicity Category IV) to male and female Sprague-Dawley rats by the inhalation route (Rittenhouse and Wong, 1979, MRID 00015307). A 4-hour exposure to 61.7 mg/l (nominal concentration) did not cause deaths. Tremors, ataxia and depression were observed in all animals immediately after exposure, but the animals appeared normal on the following day and during the remainder of the 14-day observation period. The weight gain of the rats was unaffected by this exposure. No gross pathological changes were noted at necropsy in the seventeen organs or tissues examined, including salivary glands, teeth and eyes (brain was not examined). Based on this study, the $LC_{50}$ value for both male and female rats is greater than 61.7 mg/l. This study is adequate.

In an earlier study, Cavalli (1970, MRID 00014684) exposed male and female Sprague-Dawley rats to vapors of RE 12420 (Orthene Technical) for one hour. The vapors were produced by passing dry air through solid crystalline or melted test material. There were no deaths or signs of toxicity and all organs, but the lungs, were normal. Animals sacrificed at 14 days showed scattered instances of pulmonary congestion and indications of chronic respiratory disease. However, because sizing of particles was not done, it was impossible to determine whether or not RE 12420 was respirable and, therefore, impossible to conclude whether or not the pulmonary findings were treatment-related. Because the concentration of RE 12420 in the inhalation

chamber was unknown, nothing can be said about the $LC_{50}$ values in this particular study. These data are of supplementary nature.



The above information is sufficient to satisfy the data requirement.

Primary Eye Irritation (163.81-4)

The minimum data requirement for primary eye irritation is one test on each manufacturing-use product and each formulated product, preferably using the albino rabbit.

This test may be waived upon submission of data demonstrating that the test substance has a pH of 1-3 or 12-14; for regulatory purposes, a test substance with a pH of 1-3 or 12-14 will be considered corrosive to the eye.

Orthene Technical was a mild eye irritant (Toxicity Category III) in the New Zealand strain rabbits. The test material was less irritating to the unwashed eyes than to the eyes which were washed with water for 15 minutes, after a 5-minute exposure (Narcisse and Cavalli, 1971, MRID 00014686). However, such a prolonged washing procedure could have enhanced the irritation potential of the test material.

Two rabbits (out of six) with washed eyes had mild corneal opacity and iritis for the first two days after exposure, but none occurred in rabbits with unwashed eyes. Conjunctival redness and discharge were present in both treatment groups, in most rabbits, on day three and in one rabbit on day seven after exposure. All eyes were clear on day 14. This study is adequate.

In another study (Levy and Wong, 1979, MRID 00015306), Orthene 97% Technical (Orthene Specialty Concentrate) produced slight conjunctival redness, discharge and chemosis in most of the rabbits (New Zealand strain) immediately after exposure. At 24 hours, the eyes of all rabbits were free from irritation. There was no corneal opacity or iritis in both treatment groups. In this study, the eyes of three rabbits were washed at 30 seconds after instillation of the test material and the washing time was one minute. However, the dose used (31.8 mg) was considerably smaller than the required 100 mg. The toxicity category cannot, therefore, be assigned to the test material used in this study. No reason was given for decreasing the size of the dose. These data are of supplementary nature.

The information is sufficient to satisfy the data requirement.

Primary Dermal Irritation (163.81-5)

The minimum data requirement for primary dermal irritation is one test on each manufacturing-use product and each formulated product, preferably using the albino rabbit.

This test may be waived upon submission of data demonstrating that the test substance has a pH of 1-3 or 12-14; for regulatory purposes, a test substance with a pH of 1-3 or 12-14 will be considered corrosive to the skin.

Orthene 97% Technical (Orthene Specialty Concentrate) was not a dermal irritant (PIS = 0.1; Toxicity Category IV) to New Zealand female rabbits (Levy and Wong, 1979, MRID 00015305). At 24 hours after a 24-hour exposure, two

rabbits had a well-defined erythema on both intact and abraded sites. At 48 hours, a very slight erythema was still present on the intact back of one rabbit. At 72 hours, all rabbits were free from irritation. This study is adequate.

The above information is sufficient to satisfy the data requirement.

Dermal Sensitization (163.81-6)

The minimum data requirement for dermal sensitization is an intradermal test for each manufacturing-use product and each formulated product, preferably using the guinea pig.

Available data on dermal sensitization are of supplementary nature only and do not permit conclusion whether or not Orthene Technical is a dermal sensitizer. These data were obtained from topical applications of small volumes of highly diluted Orthene Technical (RE 12420). A comment is made that the concentration showing the minimal amount of irritation was used in the study. However, daily scores for virtually all rabbits and guinea pigs were zero and concentrations higher that 1% or intradermal injections were not tried.

On one study (Cavalli, 1970, MRID 00014691), male New Zealand rabbits were exposed daily for 16 days to 0.1 ml of 1% RE12420, dissolved in acetone. Ten days after the exposure, the rabbits were challenged with the same dose of RE 12420 and were observed for 24 hours. The test material was applied on the

shaved area over the right shoulder and the challenge dose was placed "at a remote site."

RE 12420 was not a sensitizer under the conditions of this test. The average combined Draize scores after the initial applications and the challenge dose were 0.1 and 0.3, respectively.

In another study (Ebbens, 1971, MRID 00014692), guinea pigs were treated wth Webril pads containing Orthene Technical (0.1 ml of 1% "dilution") every other day for a total of ten times. The challenge dose (two Webril pads) was given two weeks later. A 0.05% (w/v) solution of chlorodinitrobezene in ethanol was used as a positive control.

Chlorodinitrobenzene produced mild erythema after the challenge dose. Orthene Technical was not a sensitizer under the conditions of this test. There was no edema or erythema at all scoring times. However, this study does not completely meet the requirements of 163.81-6 in both design and reporting. The 0.1 ml dose used is too small for topical patch applications. No mention is made of the trial tests which led to the selection of 1% aqueous dilution of Orthene as a nonirritating concentration. The age and sex of animals used are not mentioned.

The above information is not sufficient to satisfy the date requirements; additional testing is required.

Acute Delayed Neurotoxicity (163.81-7)

003122

The minimum data requirement for acute delayed neurotoxicity is one test for the technical chemical, using the adult hen.

An acute delayed neurotoxicity test is required if the active ingredient, or any of its metabolites, degradation products, or impurities cause esterase depression or are structurally related to a substance that induces delayed neurotoxicity.

It could not be determined from this study (Chevron Chemical Company, 1979, MRID GS0042-005) whether or not Orthene Technical caused delayed neurotoxicity.

Seven-month old hens received single oral doses of Orthene Technical (375 mg/kg) and single i.m. doses of atropine (5 mg/kg). The positive controls received single oral doses of triorthocresol phosphate (TOCP; 500 mg/kg). The hens were observed for neurotoxic signs for 21 days.

Delayed neurotoxicity (leg paralysis) was reported for all of the TOCP-treated hens, but for none of the Orthene-treated hens. Gross necropsy and histopathology of the sciatic nerve and spinal cord revealed no abnormalities in the Orthene-treated hens.

The following deficiencies were noted in this study: 1) hens were too young; 2) challenge dose was not administered; 3) brain tissue was not examined histopathologically; and 4) axon-specific staining for observing possible

axonal degeneration was not successfully used. Data obtained from this study are of supplementary nature.

The above information is not sufficient to satisfy the data requirement; additional testing is required.

Special Acute Studies: intraperitoneal $LD_{50}$, potentiation of acute oral toxicity and cholinesterase inhibition.

There are currently no specific data requirements under 40 CFR 163 for these studies.

A. Intraperitoneal $LD_{50}$ (rat).

In this study (Coquet, 1972, MRID 00014935), male Sprague - Dawley rats were injected with 750, 1000, 1250 or 1500 mg of RE 12420 (Orthene) Technical/kg of body weight. Female rats received the test material only at the 1000 mg/kg level. The animals were observed for 14 days.

The $LD_{50}$ value for male rats was 1103 mg/kg. The following toxic signs were reported: 1) slight tremors of short duration at the f750 mg/kg level; and 2) pronounced tremors and slight ocular and nasal hemorrhages lasting 5-7 days at the 1000 - 1500 mg/kg levels. This study is acceptable.

B. Potentiation of acute oral toxicity (rat and mouse).

The purpose of these studies was to determine if the acute oral toxicity

of Orthene Technical was potentiated by the following cholinesterase-inhibiting insecticides: Azinphos Methyl, Carbaryl, Malathion, Methyl Parathion, Oxydemeton Methyl, Monitor, Macbar, Fenitrothion and Vamidothion. Positive results were obtained in rats with the first five pesticides (Rittenhouse et. al., 1972, MRID 00014933), but Monitor was essentially without effect (Kretchmar, 1972, MRID 00014934). No synergism (potentiation) was observed in mice in the case of Carbaryl, Macbar, Fenitrothion or Vamidothion (Kobayashi, 1978, MRID 05019361).

The potentiation studies were conducted in two phases. First the $LD_{50}$ value on the basis of active ingredient for each of the test materials was determined experimentally. In the second phase, the $LD_{50}$ values for the mixtures of Orthene in combination with other insecticides were calculated, assuming strictly additive toxicity. These mixtures were then fed to the animals in order to obtain the experimental $LD_{50}$ values. The degree of synergism (potentiation) was determined by dividing the theoretical $LD_{50}$ value for a mixture by the experimental $LD_{50}$ value for same mixture. A ratio greater than 1.0 indicated the extent of potentiation.

In one study with adult male Sprague-Dawley rats, the acute oral $LD_{50}$ for Orthene Technical was 1230 mg/kg, expressed as acephate (Rittenhouse et. al., 1972, MRID 00014933). All of the experimental $LD_{50}$ values for the equitoxic mixtures of Orthene and a test substance were lower than the theoretical $LD_{50}$ values for the same mixtures, indicating potentiation. As is shown below, the extent of potentiation was greatest with Carbaryl and smallest with Methyl Parathion.

| Orthene + Test Material | Ratio Theoret. $LD_{50}$ / Exptl. $LD_{50}$ |
|---|---|
| Azinphos Methyl | 1.9 |
| Carbaryl | 3.9 |
| Malathion | 2.8 |
| Methyl Parathion | 1.6 |
| Oxydemeton Methyl | 1.9 |

003122

The equitoxic mixtures caused typical signs of cholinesterase inhibition. Gross necropsy did not revealed changes which could be attributed to any of test materials. This study is acceptable.

The acute oral $LD_{50}$ values obtained in another potentiation study (Kretchmar, 1972, MRID 00014934) with young Charles River rats are summarized below:

| Test Material | $LD_{50}$ (mg/kg) |
|---|---|
| Orthene Technical | 2025 |
| Monitor Technical | 21.15 |
| Orthene + Monitor* | 1023 (Theoretical) |
| Orthene + Monitor* | 813.2 (Experimental) |

*Equitoxic mixtures.

The ratio of theoretical $LD_{50}$ (1023 mg/kg) to experimental $LD_{50}$ (813.2 mg/kg) equals 1.25, indicating that the acute oral toxicity of Orthene was potentiated insignificantly by Monitor. However, these data are based on only two rats/sex/level and are, therefore, of supplementary mature.

Kabayashi (1978, MRID 05019361) studied the synergism in the acute oral toxicity of Orthene Technical (acephate) in combination with carbamates (Carbaryl or Macbar) and organophosphates (Fenitrothion or Vamidothion), in male and female mice. These data are summarized below.

| Pesticide Combination Ratio | Experimental LD50* | Theoretical LD50* | Degree of Synergism** |
|---|---|---|---|
| Acephate | 735 | | |
| | 687 | | |
| Acephate:Macbar | | | |
| (2:1) | 600 | 459 | 0.77 |
| | 687 | 412 | 0.60 |
| (1:1) | 350 | 386 | 1.10 |
| | 458 | 344 | 0.75 |
| (1:1.5) | 525 | 353 | 0.67 |
| | 458 | 344 | 0.75 |
| Macbar | 262 | | |
| | 229 | | |
| Acephate | 504 | | |
| | 566 | | |
| Acephate:Carbaryl | | | |
| (2:1) | 642 | 436 | 0.68 |
| | 600 | 449 | 0.75 |
| (1:1) | 490 | 406 | 0.83 |
| | 561 | 406 | 0.72 |
| (1:1.5) | 449 | 393 | 0.88 |
| | 566 | 385 | 0.68 |
| Carbaryl | 344 | | |
| | 317 | | |

| | | | |
|---|---|---|---|
| Acephate | 794<br>794 | | |
| Acephate:Fenitrothion | | | |
| (1:1) | 1414 | 1017 | 0.72 |
| | 1260 | 1017 | 0.81 |
| (1:2) | 1414 | 1122 | 0.79 |
| | 1782 | 1122 | 0.83 |
| Fenitrothion | 1414<br>1414 | | |
| Acephate | 635 | | |
| Acephate:Vamidothion | | | |
| (1:1) | 80 | 84 | 1.05 |
| Vamidothion | 45 | | |

*$LD_{50}$ = mg/kg of body weight. In each column, the upper figure is for male and the lower for female mice.

** Ratio of theoretical $LD_{50}$ to experimental $LD_{50}$.

These data show that treatment of male and female mice (ICR strain) with single doses of acephate and an organophosphate or a carbamate did not cause potentiation of the acute oral toxicity of the mixtures studied. The ratios of theoretical $LD_{50}$ values to experimental $LD_{50}$ values ranged from 0.60 to 1.10, indicating no synergism. The $LD_{50}$ values for Orthene Technical (expressed as acephate) ranged from 504 mg/kg to 794 mg/kg, placing the product in Toxicity Category III. This study is acceptable.

Additional potentiation studies are not needed.

C. Cholinesterase inhibition

The effects of acute oral and inhalation exposures to RE 12420 (Orthene Technical) on the cholinesterase activities in rat plasma and erythrocytes

were studied by Cavalli in 1970 (MRID 0014678 and MRID 00014685). Data 003122 obtained in these studies are only of supplementary nature. Cholinesterase activity in brain tissue was not studied.

In one study (Cavalli, 1970, MRID 00014678), 4 male Sprague-Dawley rats were intubated with single doses of RE 12420 (900 mg/kg) and were sacrificed after 20-30 minutes of exposure. Parathion 4E (15 mg/kg) was used as a positive control.

At the time of sacrifice, all treated rats showed tremors, rhinorrhea and salivation. Plasma cholinesterase activity was inhibited an average of 3% (range:0-9%) in the Parathion-tested group and 16% (range:11-25%) in the RE 12420-treated group, which is insignificant. The erythrocyte cholinesterase activity was inhibited an average of 61% (range:47-69%) in the Parathion-treated rats, but the inhibition varied greatly in the RE 12420-treated rats. Specifically, cholinesterase activity was inhibited 2, 58, 61 and 29% in rats No. 1, 2, 3, and 4, respectively. Because only one exposure level and too few animals were used in this study, and because cholinesterase activities varied so greatly, it is impossible to determine the extent of inhibition even at this single level of RE 12420 tested. Not much can, therefore, be learned from this study about the effects of acute oral exposure to RE 12420 on cholinesterase activities in the rat. These data can, at best, be considered only as supplementary.

In another study (Cavalli, 1970, MRID 00014685), 5 female Sprague-Dawley rats were exposed to vapors of RE 12420 for 4 hours. The vapors were generated by passing dry air through crystalline RE 12420.

There were no toxic signs or mortality, and plasma and erythrocyte cholinesterase activities were not inhibited. However, the RE 12420 particles were not sized and so it is unknown whether or not they were respirable. The concentration of the test material in the inhalation chamber was also not determined. These data are, therefore, of supplementary nature.

Testing for cholinesterase activity in acute studies is not required.

Subchronic Oral Toxicity (163.82-1)

The minimum data requirement for subchronic oral toxicity is one test for the technical chemical in two mammalian species, preferably using the rat and dog.

A subchronic oral toxicity test is required if pesticidal use requires a tolerance, an exemption from a tolerance, the issuance of a food additive regulation, or is likely to result in repeated human exposure through the oral route.

A separate subchronic oral toxicity study with rats is not available. However, a 90-day rat feeding/cholinesterase inhibition study is available (Plank, 1972, MRID 00014832). A 28-month rat feeding/oncogenic study has also recently been submitted (Chevron Chemical Company, 1981, MRID GS0042-011). Adequate data are, therefore, available to meet the requirement of subchronic oral testing with rats.

A separate dog subchronic oral toxicity study is unavailable. However, there exists a valid 2-year dog feeding study (Chevron Chemical Company, 1972, MRID

GS0042-006). Adequate data are, therefore, available to meet the requirement for subchronic oral testing with dogs.

Dosing crossbred pigs, 2 males and 2 females/level, with 3, 10, or 30 ppm of acephate and 0.6, 2 or 6 pm of Ortho 9006, respectively, for 21, 27 or 30 days caused no adverse behavioral reactions, mortality or adverse gross pathology (Ladd, 1972, MRID 00015227). However, all test groups exhibited lower food consumption when compared to controls. There was also a downward trend in body weight gains with increased exposure levels. The NOEL was, therefore, less than 3 ppm of acephate and 0.6 ppm of Ortho 9006.

The test material fed was a 5:1 mixture of acephate and methamidophos (Ortho 9006, Monitor). The experimental diets were prepared by adding this mixture to the stock diet. This study is acceptable.

In another study (Fletcher, 1972, MRID 00014830), white leghorn chickens, 4 males and 25 females/level, were fed diets containing 3, 10 or 30 ppm of Orthene Technical for 92 days, and were observed for 28 days. This treatment produced no toxic symptoms and did not affect mortality, food consumption or body weight gain of the birds. The NOEL was, therefore, greater than 30 ppm.

The above information is sufficient to satisfy the data requirement.

Subchronic Oral Toxicity (163.82-1): Cholinesterase Inhibition

According to the proposed Guidelines 40 CFR 163.82-1, if the test substance contains a carbamate, an organophosphate or any chemical that produces

acetylcholinesterase inhibition, the enzyme activity for plasma, red blood cell and brain should be monitored. In the pre-Guideline era and occasionally now, too, separate studies of cholinesterase activity have been conducted and submitted to the regulatory agencies. Four such studies with RE 12420 Technical (Orthene) containing acephate (an organaphosphate) as an active ingredient are discussed below.

In one study (Cavalli, 1970, MRID 00014687), female Sprague-Dawley rats, 15/dosage, were intubated daily with 30, 100 or 1200 ppm of RE 12420 for 21 days. Monitor, 10 ppm, was used as a positive control. On test days 7, 14, and 21, 5 rats from each group were sacrificed and cholinesterase activities were determined in plasma and erythrocytes.

Toxic symptoms were not seen in any of the animals fed RE 12420 or Monitor. The cholinesterase activity data are summarized below.

Inhibition of cholinesterase activity in plasma (%)

| Test Week | RE 12420, ppm | | | Monitor, ppm |
|---|---|---|---|---|
| | 30 | 100 | 1200 | 10 |
| 1 | 0 | 14 | 24* | 16 |
| 2 | 6 | 12 | 28* | 18 |
| 3 | 0 | 10 | 24* | 21 |

Inhibition of cholinesterase activity in erythrocytes (%).

| Test Week | RE 12420, ppm | | | Monitor, ppm |
|---|---|---|---|---|
| | 30 | 100 | 1200 | 10 |
| 1 | 3 | 9 | 37* | 28* |
| 2 | 21** | 18** | 37* | 26* |
| 3 | 15** | 21* | 45* | 33* |

*P=0.01; **P=0.05; 10, 30, 100 and 1200 ppm correspond to 0.5, 1.5, 5.0 and 60 mg of the test material/kg of body weight, respectively.

According to these data, plasma cholinesterase activity was inhibited significantly only in the 1200 ppm group and erythrocyte cholinesterase activity was inhibited in all test groups, when compared to controls. Monitor was a more potent inhibitor of cholinesterase activity than RE 12420. The NOEL was not determined in this study. Based on the inhibition of plasma cholinesterase activity, the NOEL was 30 ppm (1.5 mg/kg). Based on the inhibition of erythrocyte cholinesterase activity, the NOEL was less than 30 ppm. This study is acceptable.

In another study (Plank, 1972, MRID 00014832), male and female Charles River rats, 15 or 35/sex/dose, were fed 1, 5, 30, 100 or 300 ppm of Ortho 12420 Technical (Orthene) for 90 days. The 30, 100 and 300 ppm groups had a 4-week recovery period, but the first two groups had none.

Dietary levels of Ortho 12420 Technical greater than 30 ppm inhibited plasma cholinesterase activity at all sampling times in male rats. The inhibition varied from 24% to 68% during the treatment, but was only 7-15% during the 4th week of the recovery period. There was no inhibition at the 1 and 5 ppm levels. Similar results were obtained for the female rats. It is perhaps noteworthy that values for the plasma cholinesterase activity of female controls were about 2-4 times greater than those reported for male controls.

Erythrocyte cholinesterase activity was depressed 0-13% in both male and female rats in the 1, 5 and 30 ppm groups. There was no inhibition during the first week of the recovery period. Both male and female rats fed 100 or 300 ppm of the test material showed an 11-28% depression in the erythrocyte cholinesterase activity, with most of the values below 20%.

Brain cholinesterase activity was essentially unaffected in male and female rats at the 1 and 5 ppm levels, with most of the inhibitions being below 10%. Brain cholinesterase activities in the 30, 100 and 300 ppm groups were inhibited 25-37%, 43-60% and 55-80%, respectively, during the test period, but only 10-14% during recovery week 4.

The NOEL was, therefore, 5 pm (0.25 mg/kg), based on the inhibition of cholinesterase activity in plasma, erythrocyte and brain, in male and female rats. The LEL was 30 ppm (1.5 mg/kg). A 25-45% inhibition of cholinesterase activity in brain and plasma, in male and female rats, was observed at that level. Toxic signs, if any occurred, were not reported. This study is adequate.

Cholinesterase activity was also studied in adult human subjects, 7 males and 7 females, during and after a subchronic oral exposure to RE 12420 and RE 9006 (Garofalo, 1973, MRID 00015160). RE 9006 (Monitor) and RE 12420 (Orthene) were fed as mixtures in ratios of 1:4 or 1:9 until plasma and/or erythrocyte cholinesterase inhibition occurred. The 1:9 Monitor:Orthene group was fed 0.1, 0.2, 0.3 and 0.4 mg of the mixture/kg of body weight. The 1:4 Monitor: Orthene group received only 0.1 and 0.2 mg/kg levels. Each dose could be fed for a maximum of 21 days.

All subjects were in good health at the completion of the study. Erythrocyte cholinesterase activity was not inhibited during the 37-73 days of feeding the test materials. Data for plasma cholinesterase inhibition are summarized below.

| Test Material Combination Monitor: Orthene | NOEL (mg/kg/day) | | LEL (mg/kg/day) |
|---|---|---|---|
| 1:4 | 0.1 | M + F | 0.2 |
| 1:9 | 0.2 | M | 0.3 |
| | 0.3 | F | 0.4 |

Plasma cholinesterase activities were inhibited significantly in the 1:4 and 1:9 (Monitor:Orthene) groups. The inhibition in the 1:4 group was first noted at the 0.2 mg/kg level after 16 days of dosing at that level and occurred in all subjects studied (2 males and 2 females). The first significant inhibition in the 1:9 group was observed at the 0.3 mg/kg level after 21 days of dosing at that level and only in the male subjects. The first significant inhibition in the female subjects was noted at the 0.4 mg/kg level after 10 days of dosing at that level. All depressed cholinesterase activities returned to the pretest levels during the 7-day observation period. The inhibition of cholinesterase activity was considered significant when it "was greater that two standard deviations below mean pretest activity for two consecutive bleedings."

The pretest cholinesterase activities in the erythrocytes were about the same in males and females. However, nearly all plasma cholinesterase activities in the female subjects were about one-half of those reported for the males.

The results from this study are acceptable as supplementary data. Unsupervised weekend dosing and lack of urine analysis for the test materials constitute weak points in this study.

Cholinesterase activities in the rat plasma and RBC, inhibited by Technical Orthene, returned to the control values within a week after the Orthene feeding was discontinued (Bio/dynamics Inc., 1989, MRID GS0042-012). The recovery from cholinesterase inhibition in the brain tissue was rapid initially and slow thereafter. During the first week of the recovery period, the inhibition of brain cholinesterase activity decreased from 34% to 8.7%, but the enzyme was still inhibited 5.5% during recovery week 6.

This study consisted of two parts and both were conducted concurrently. In Part I, 43-day old male Sprague-Dawley rats were fed a diet containing 75 ppm (7.5 mg/kg) of Technical Orthene (92.8% pure) for 6, 11, 15 or 20 days, in order to study the cholinesterase inhibition profile. As can be seen from the data below, Orthene Technical was a more potent inhibitor of brain cholinesterase activity than of plasma and RBC cholinesterase activities.

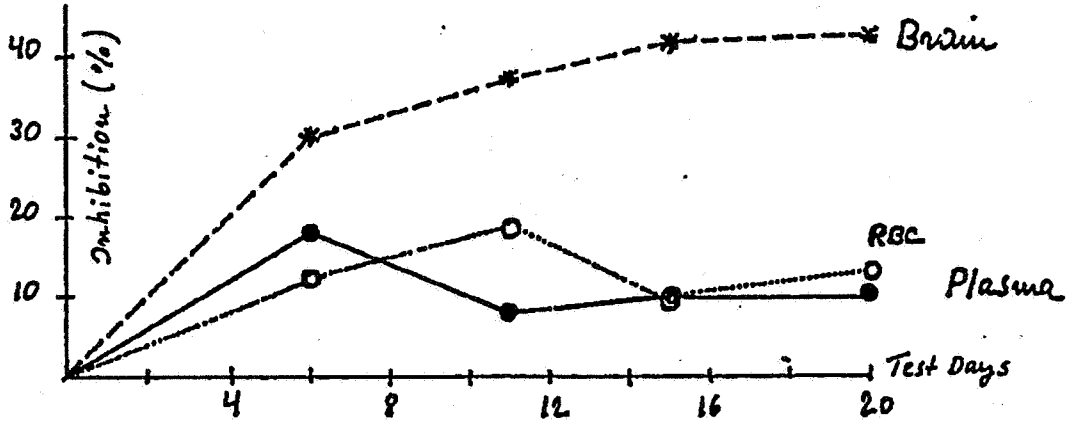
Percent Inhibition of Cholinesterase Activity in Part I of the Study

| Test Day | Plasma | RBC | Brain |
|---|---|---|---|
| 6 | 18.2** | 11.5* | 30.4** |
| 11 | 8.3 | 17.9 | 37.3** |
| 15 | 10.0 | 8.7 | 40.9** |
| 20 | 10.0 | 13.0 | 41.5** |

Significantly different from control: * $p \leq 0.05$;
** $p \leq 0.01$

Graphic representation of these data (Figure 1) shows that the plasma and RBC cholinesterase activities became inhibited maximally after 6 and 11 days of feeding Orthene, respectively, and declined thereafter, even though the Orthene feeding was continued for a total of 20 days. In the case of brain cholinesterase activity, the enzyme was inhibited rapidly at first and then slowly until the termination of the test.

Figure 1. Effect of Orthene Feeding on Cholinesterase Activity




In part II of the study, rats were fed a diet containing 75 ppm of Technical Orthene for 7 days, that is, for as long as it took to achieve a 25% or greater inhibition of brain cholinesterase activity. A 30.4% brain cholinesterase depression was actually observed in Part I on test day 6 and was confirmed in Part II on test day 7. On test day 8, the remaining Part II Orthene-treated rats were placed on the Orthene-free (control) diet for a maximum of 6 weeks (recovery period). As can be seen from the data below, the inhibited cholinesterase activity in plasma and RBC returned quickly to control levels after the feeding of Orthene was discontinued. A rapid but partial recovery (91%) of brain cholinesterase activity was observed within 7 days of feeding an Orthene-free diet. Brain cholinesterase activity was still inhibited 5.5% during recovery week 6, indicating that this enzyme returns slowly to normal (control) levels.

Percent Inhibition of Cholinesterase Activity in Part II of the Study

| | Test Day 7 | Recovery Weeks 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|
| Plasma | 25.0** | 0 | 0 | 0 | 0 |
| RBC | 21.4** | 0 | 4.8 | 10.5 | 0 |
| Brain | 34.0** | 8.7* | 8.2** | 2.3 | 5.5 |

Significantly different from control: * $p \leq 0.05$; ** $p \leq 0.01$

This study is acceptable:

The above information is sufficient to satisfy the data requirement.

Subchronic 21-day Dermal Toxicity (163.92-2)

The minimum data requirement for subchronic 21-day dermal toxicity is one test for the technical chemical, preferably using the albino rabbit.

A subchronic 21-day dermal toxicity test is required if pesticidal use is likely to result in repeated human skin contact.

A 21-day dermal toxicity study with Orthene Technical is missing and this is considered a toxicity data gap. The widespread use of Orthene both commercially and noncommercially could readily result in repeated human skin contact.

The above information is not sufficient to satisfy the data requirement; additional testing is required.

Subchronic 90-day Dermal Toxicity (163.82-3)

The minimum data requirement for subchronic 90-day dermal toxicity is one test for the technical chemical, preferably using the albino rabbit.

The subchronic 90-day dermal toxicity is required if pesticidal use will involve purposeful application to the skin or will result in human exposure comparable to that, for example, from swimmming pool additives or pesticide-impregnated fabrics.

This study is not required.

003122

Subchronic Inhalation Toxicity (163.82-4)

The minimum data requirement for subchronic inhalation toxicity is one test for the technical chemical, preferably using the laboratory rat.

A subchronic inhalation toxicity test is required if pesticidal use may result in repeated inhalation exposure at a concentration that is likely to be toxic, as determined from results of acute inhalation testing.

This study is not required.

Subchronic Neurotoxicity (163.82-5)

The minimum data requirement for subchronic neurotoxicity testing is one test for the technical chemical, using either the adult hen or a mammalian species.

A subchronic neurotoxicity test is required if the pesticide has shown positive results in the acute delayed neurotoxicity test or induced irreversible neurological toxicity in a mammalian species.

It cannot be stated with certainty whether or not this test is required because data reported in the acute delayed neurotoxicity study (Chevron Chemical Company, 1979, MRID GS0042-005) are of supplementary nature. Although Orthene Technical (375 mg/kg) did not cause delayed neurotoxicity in that study, 7-month old hens were used, or younger than the test requires, and

a challenge dose was not administered. However, no paralysis or other neurological symptoms were observed in the subchronic oral feeding study with adult humans (Garofalo, 1973, MRID 00015160), a very sensitive species.

Chronic Feeding (163.83-1)

The minimum data requirement for chronic feeding is one test for the technical chemical, preferably using the laboratory rat.

A chronic feeding study is required if pesticidal use requires a tolerance, exemption from a tolerance, issuance of a food additive regulation, or is likely to result in repeated human expsoure over a significant portion of the life-span.

Two chronic feeding studies have been conducted, a 2-year dog feeding study (Hartke, 1971, MRID 00014699; Chevron Chemical Company, 1972, MRID GS0042-006) and a 28-month rat feeding/oncogenic study (Bio/dynamics Inc., 1981, MRID GS 0042-011).

Groups of 4 beagle dogs/sex were fed Orthene Technical at the dietary levels of 10, 30 or 100 ppm for 2 years. There was no effect on mortality, body and organ weights, food consumption, behavioral reactions, urinalysis, hematology, blood chemistry, gross necropsy and histopathology. Differences from controls in hematology and blood chemistry, exhibited once or twice by one female in the 10 ppm group and one male in the 100 ppm group, were not considered Orthene-related. At the 100 ppm level, erythrocyte cholinesterase activity was inhibited 29-79% in both sexes and brain cholinesterase activity

(determined only at terminal sacrifice) was inhibited about 27% in two females. No other significant depressions in plasma, erythrocyte or brain cholinesterase activities were noted. The NOEL was, therefore, 30 ppm (0.75 mg/kg) and the LEL was 100 ppm (2.5 mg/kg), based on the inhibition of brain, plasma and erythrocyte cholinesterase activities, for males and females. The 100 ppm level was also a NOEL for systemic toxicity, for males and females. This study is adequate.

Groups of 75 Sprague-Dawley rats/sex were fed Technical RE 12420 (Orthene; a.i. = 92.4%) at the dietary levels of 0, 5, 50 or 700 ppm (nominal concentrations) for 28 months (Bio/dynamics Inc., 1981, MRID GS0042-011). The following results were obtained:

1. There was no effect on mortality, hematology, clinical chemistry, urinalysis, and organ weights at all levels tested, in males and females.

2. Body weights of high-dose (700 ppm) males were 4-18% less than controls throughout the study, even though their food intake was increased by 4-8% above that of the controls. Body weights of females at all dose levels and of males at the 5 and 50 ppm levels were similar to those of controls.

3. Food intake was initially greater than that of controls in all groups of females. This increased consumption persisted at 4-24% throughout the test for the 700 ppm group, but returned to normal in the 5 ppm group before the 6th week, and before the 18th week in the 50 ppm group.

4. Hyperactivity was observed in some of the animals, especially the

high-dose males, during the first six months of the study. The hyperactivity was accompanied in the males by increased aggressiveness. For example, at test week 16, 23 rats (31%) in the high-dose group were aggressive. The incidence of aggressiveness in the 0, 5 and 50 ppm groups was 5, 13 and 13%, respectively.

5. An increased incidence of focal retinopathy was observed in the high-dose males and females in comparison to that seen in the rest of the test animals. The retinopathy was identical to the effects of the sialodacryoadenitis virus infection which is commonly seen in laboratory rats and was known to be present in these test animals. The increased incidence in the high-dose animals may have been fortuitous, or it may have been due to an increased susceptibility related to the dose.

6. An increased incidence of renal calculi was seen at autopsy in the high-dose females compared to the controls. No information is given on the compositions of the calculi nor are data available for statistical analysis of the incidence.

7. The most common neoplasms were found in the pituitary gland. The incidence did not vary between the treatment levels or controls, but the neoplasms were more frequent throughout in the females. The next most common neoplasms were in the mammary glands of the females, and they were not dose-related.

8. There was a higher incidence of adrenal medullary tumors in the treated males than in the control males. The reported incidences were: controls,

2/75 (2.7%); 5 ppm level or low-dose, 7/72 (9.7%); 50 ppm or mid-dose, 11/71 (15.5%); and 700 ppm or high-dose, 9/74 (12.2%). All of these neoplasms, except two, were reported as "benign (adrenal medullary adenomas)." The remaining two were classified as "malignant (adrenal medullary carcinomas)." These neoplasms were apparently pheochromocytomas (memo from Mr. D. F. Dye, Chevron Chemical Company, to Mr. W. H. Miller, EPA/RD, 4/16/82; attached to the Data Evaluation Record). The testing laboratory cited the historic incidence of medullary neoplasms in control rats in their own laboratory as ranging from 0.9% to 15%. These data have not yet been submitted for evaluation.

9. Plasma and erythrocyte cholinesterase activities were significantly decreased ($p \leq 0.01$) at most sampling times in the high-dose males and females. Similar results were found at some sampling times in the mid-dose animals. The plasma and erythrocyte cholinesterase values for the low-dose groups were not significantly below those of the controls. These data are summarized below.

Percent Inhibition of Plasma Cholinesterase Activity

in Rats Fed Technical Orthene

| Dose ppm | Sex | Sampling Times: Test Weeks 6 | 7 | 19 | 28 | Test Months 12 | 18 | 22 | 24 | 28 |
|---|---|---|---|---|---|---|---|---|---|---|
| 5 | M | *** | | | | | | | | |
| | F | 6 | 19 | | | | | | 6 | 10 |
| 50 | M | | 29 | | 9 | 21 | 8 | 24 | 17 | |
| | F | 38** | 36** | 13 | 13 | 16 | 16 | | 19 | 29** |
| 700 | M | 10 | 41 | 29** | 27** | 21 | 54 | 29 | 25 | 50** |
| | F | 72** | 68** | 57** | 58** | 58** | 56** | 52** | 50** | 57** |

Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$
***Blank spaces denote no inhibition.

Percent Inhibition of Erythrocyte Cholinesterase Activity in Rats Fed Technical Orthene

| Dose ppm | Sex | Sampling Times: Test Weeks 6 | 7 | 19 | 28 | Test Months 12 | 18 | 22 | 24 | 28 |
|---|---|---|---|---|---|---|---|---|---|---|
| | *** | | | | | | | | | |
| 5 | M | 13 | 9 | | 4 | 22 | | | | |
| | F | | 29 | | | 3 | | | 8 | 4 |
| 50 | M | 3 | 18 | 13* | 13* | 13 | 31 | | 10 | 26* |
| | F | 25* | 42 | 11 | 13 | 10 | | 19 | 15 | 21** |
| 700 | M | 41** | 41 | 47** | 67** | 50** | 39* | 21 | 23 | 57** |
| | F | 44** | 58* | 44** | 61** | 38** | 21 | 37 | 33** | 46** |

Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$

***Blank spaces denote no inhibition.

10. There was a dose-related inhibition of brain cholinesterase activity for all high- and mid-dose animals at all sampling times. At the 50 and 700 ppm levels, the inhibition was 33-45% and 66-83%, respectively. The inhibition was low (0-13%) at the 5 ppm level. These data are summarized below.

Percent Inhibition of Brain Cholinesterase Activity in Rats Fed Technical Orthene

| Dose ppm | Sex | Sampling Times: Test Weeks 7 | 19 | Test Months 12 | 22 | 28 |
|---|---|---|---|---|---|---|
| 5 | M | 9* | 4 | 13** | 0 | 11** |
| | F | 13 | 11** | 5 | 1 | 9* |
| 50 | M | 35** | 34** | 41** | 43** | 40** |
| | F | 45** | 37** | 33 | 40** | 37** |
| 700 | M | 77** | 69** | 70** | 69** | 71** |
| | F | 83** | 73** | 71** | 66** | 69** |

Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$

Based on the inhibition of cholinesterase activity in plasma, erythrocytes and brain, the NOEL is 5 ppm (0.25 mg/kg) and the LEL is 50 ppm (2.5 mg/kg) for male and female rats. This study is adequate as a chronic feeding study.

The above information is sufficient to satisfy the data requirement.

Oncogenicity (163.83-2)

The minimum data requirement for oncogenicity is testing in two mammalian species, preferably the rat and mouse, using the technical chemical.

An oncogenicity test is required if the active ingredient, or any of its metabolites, degradation products or impurities is/are structurally related to a recognized carcinogen or causes a mutagenic effect; requires a tolerance or an exemption from a tolerance, issuance of a food additive regulation, or is likely to result in repeated human exposure over a significant portion of the life-span.

The oncogenic potential of Orthene Technical has been studied in the mouse (Chevron Chemical Company, 1979, MRID GS0042-003; Chevron Chemical Company, 1981, MRID GS0042-002) and in the rat (Bio/dynamics Inc., 1981, MRID GS0042-011). Only interim reports are presently available for the mouse study.

RE 12420 Technical (Orthene) did not show oncogenic properties during the first 12 months of feeding to mice (CD-1 strain) at dosages of 0, 50, 250 and 1000 ppm (Chevron Chemical Company, 1979, MRID GS0042-003). The 50 ppm (7.5

mg/kg) and 250 ppm (37.5 mg/kg) levels had no effect on the gross necropsy, histopathology, appearance, behavior, mortality, weight gain and food consumption of the animals. One alveolar cell adenoma in a male mouse, non-neoplastic enlargement of hepatocytes, weight loss (14-25%) and decreased food intake (16-27%) was reported for the 1000 ppm level. A total of 100 mice were examined by gross necropsy and 80 mice were examined histopathologically during the initial 12 months of study.

A preliminary draft of the histopathology findings for the controls and the 1000 ppm (highest tested) group, for the entire duration of this study (2 years), has subsequently been submitted (Chevron Chemical Company, 1981, MRID GS0042-002). The following observations were reported:

1. The incidence of malignant lymphomas and reticulum cell sarcomas in the control females and the incidence of Harderian gland adenomas in the control males and females was 4-5 times higher than that in the experimental group.

2. The incidence of hepatocellular carcinomas and adenomas in the female mice was higher in the treatment group than in the control group. Specifically, there were 12 hepatocellular carcinomas and 3 adenomas in the experimental females, but only 1 hepatocellular carcinoma and no adenomas in the control females. As far as the male mice are concerned, 4 hepatocellular carcinomas each were detected in the control and experimental groups.

In order to assess an oncogenic potential of Orthene more correctly, 003122 tissues from the 50 ppm and 250 ppm groups are currently undergoing histopathological evaluation.

Because the mouse data are incomplete, they are regarded as supplementary.

The rat (Sprague-Dawley) chronic feeding oncogenic study was conducted for 28 months, using 5, 50 or 700 ppm of Technical RE 12420 (Orthene) as the exposure levels (Bio/dynamics Inc., 1981, GS 0042-011). The following results were obtained:

1. The most common neoplasms were found in the pituitary gland. The incidence did not vary between the treatment levels or controls, but the neoplasms were more frequent throughout in the females. The next most common neoplasms were in the mammary glands of the females, and they were not dose related.

2. There was a higher incidence of adrenal medullary tumors in the treated males than in the control males. The reported incidences were: controls, 2/75 (2.7%); 5 ppm level or low-dose, 7/72 (9.7%); 50 ppm or mid-dose, 11/71 (15.5%); and 700 ppm or high-dose, 9/74 (12.2%). All of these tumors, except two, were reported as "benign (adrenal medullary adenomas)." The remaining two were classified as "carcinomas of the adrenal medulla." These neoplasms (tumors) were apparently pheochromocytomas (memo from Mr. D. F. Dye, Chevron Chemical Company, to Mr. W. H. Miller, EPA/RD; 4/16/82; attached to the Data Evaluation

Record). As is shown below, most of the tumors occurred after 22 months of study.

Incidence of Adrenal Medullary Tumors in Male Rats, Sacrificed or Found Dead, or Moribund***

| Time of Death* | Rats With Tumors | | | |
|---|---|---|---|---|
| | Dose (ppm) | | | |
| | 0 | 5 | 50 | 700 |
| 0 - 4 Months | **** | | | |
| 4-Month Sacrifice | | | | |
| 4 - 12 Months | | | | |
| 12-Month Sacrifice | | | | |
| 12 - 22 Months | | | 2 | 1 |
| 22-Month Sacrifice | | | 2 | |
| 22 - 28 Months | 2 | 2 | 6 | 2 |
| 28-Month Sacrifice | | 5 | 1** | 6** |

* Time of death is between or at scheduled sacrifices.

** One tumor at each dose was reported as carcinoma of the adrenal medulla.

*** There were 75 animals in each test group, but the reference totals have been corrected for those with adrenal tissues missing from the histopathology tissues.

**** Blank spaces denote the absence of tumors.

The incidence of adrenal medullary tumors was evaluated by several statistical tests, and the results are detailed in the Data Evaluation Record. In summary, there appeared to be a definite dose-response trend among the 0, 5, and 50 ppm groups. The 700 ppm group did not demonstrate tumor incidence that was significantly different from the 50 ppm group.

Bio/dynamics Inc. (the testing laboratory) cited the historical incidence of medullary neoplasms in control rats in their own and other laboratories as ranging from 0.9% to 20.3%. This means that the 9.7% - 15.5% incidence of medullary neoplasms, reported for the Orthene-treated male rats in this study,

falls within the "normal range" and may have nothing to do with the feeding of Orthene. The historical data will be submitted to the Agency for evaluation.

The incidence of adrenal medullary tumors in female rats was low and there were no differences between the experimental and control groups.

Adrenal medullary tumors are seldom a primary cause of death in humans. Their primary effects are alterations in the level of hormone secretion, especially epinephrine; alterations in the secretion on non-adrenal hormones, such as insulin; changes in arterial blood pressure, usually elevated; and increased incidence of numerous other diseases of metabolic or hormonal origin. All of these effects are subject to medical control.

Because the historical data on the adrenal medullary tumors in control rats have not yet been submitted for evaluation, the remaining rat oncogenic data are currently regarded as supplementary.

The above information is not sufficient to satisfy the data requirement; additional data (historical control data for the rat study) are required.

Teratogenicity (163.83-3)

The minimum data requirement for teratogenicity is testing in two mammalian species using the technical chemical.

Teratogenicity testing is required if pesticidal use requires a tolerance or an exemption from a tolerance, requires an issuance of a food additive

regulation, or is likely to result in a significant exposure to females.

Orthene Technical was not teratogenic to rat at the 200 mg/kg level (Haley, 1971, MRID 00014695) and to rabbit at the 10 mg/kg level (Chevron Chemical Company, 1980, MRID GS 0042-004). These levels were the highest ones tested.

Orthene Technical (25, 50, 100, or 200 mg/kg of maternal body weight) was fed to pregnant Charles River rats during gestation days 6-15. The parameters tested were maternal body weight, mortality and reactions; reproductive effects; fetal body weight; and fetal external, skeletal, and internal development.

Orthene was toxic to mothers at the 100 mg or 200 mg/kg levels. There were slightly more resorption sites per female at the 200 mg/kg level than in the control group (1.3 vs 0.5), but the percentage of females with resorption sites was not increased. During gestation, the weight gain of females was decreased by 25% at the 100 mg/kg level and by 29% at the 200 mg/kg level, when compared with controls. However, it is unknown whether or not the reported weights were corrected for the weights of gravid uteri.

All groups treated with Orthene Technical exhibited lacrimation following the second treatment; this reaction subsided after the fifth treatment.

Teratogenic reactions were not observed at all levels of Orthene tested. The NOEL for the teratogenic potential of Orthene in the rat is, therefore, greater than 200 mg/kg. This study is adequate.

In another study (Chevron Chemical Company, 1980, MRID GS 0042-004), pregnant Dutch Belted rabbits received orally 0, 1, 3, and 10 mg of RE 12420 Technical (Orthene)/kg of body weight during gestation days 6-27. Data from this study were also compared with earlier data obtained under the same experimental conditions with 6-aminonicotinamide, a known teratogen.

Orthene Technical was neither fetotoxic nor teratogenic under the conditions of this study. The test material did not cause maternal toxicity, except for a slight increase in nasal discharge in the 3 and 10 mg/kg groups. Two rabbits at the 10 mg/kg dosage aborted shortly before the termination of the study.

The NOEL for the teratogenic potential of Orthene in the rabbit is, therefore, greater than 10 mg/kg. This study is adequate.

The above information is sufficient to satisfy the data requirement.

Reproduction (163.83-4)

The minimum data requirement for reproduction is testing in one mammalian species, preferably the laboratory rat, using the technical chemical and lasting for two generations.

Reproduction testing is required if pesticidal use requires a tolerance or an exemption from a tolerance, requires an issuance of a food additive regulation, or is likely to result in repeated human exposure over a significant portion of the life-span.

The reproduction study is missing and this constitutes a toxicity data gap. The pesticidal use of Orthene requires a tolerance or an exemption from a tolerance, or an issuance of a food additive regulation.

The above information is not sufficient to satisfy the data requirement; additional testing is required.

Mutagenicity (163.84-1 through -4)

The minimum data requirements for mutagenicity testing will be finalized pending formulation of Agency Guidelines.

1. Gene mutation effects.

Acephate was tested in a reverse mutation plate assay, with and without a metabolic activation system, using the bacterial indicator strains Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100, and Escherichia coli WP2. Weakly positive results were seen, with and without metabolic activation for S. typhimurium TA100 and E. coli WP2. Negative results were reported for the other strains (Simmon, 1979, MRID 00028625). A reverse mutation assay was also performed in Saccharomyces cerevisiae D7 and was positive after metabolic activation and negative without activation (Mortelmans, 1980, MRID GS-0042-009).

These tests are sufficient to show that acephate can cause gene mutations in microorganisms. This effect should be examined in mammalian systems. A gene mutation assay should be performed in mammalian cells in culture

and, if positive, a mouse specific locus assay should be performed to determine if the effect is present in vivo and is heritable.

2. DNA repair effects

Acephate was tested in both the E. coli W3110/P3478 and Bacillus subtilis H17/M45 differential repair assays, and negative results were reported (Simmon, 1979, MIRD 00028625). However, these tests were classified as invalid since no toxicity was seen (acephate might not have migrated from the disc into the agar).

Acephate was shown to produce mitotic crossing-over and mitotic gene conversion in the yeast Saccharomyces cerevisiae (Mortelmans, 1980, MIRD GS-0042-009), both with and without metabolic activation.

An unscheduled DNA synthesis assay in WI-38 human fibroblast cells in culture (Simmon, 1979, MRID 00028625) showed acephate to be weakly positive without metabolic activation and negative with metabolic activation. A sister chromatid exchange assay (Evans and Mitchell, 1980, MRID GS-0042-010) showed that acephate produced sister chromatid exchanges, both with and without metabolic activation, in Chinese hamster ovary cells. Without activation, the response was greater with acephate than with the positive control.

These studies are sufficient to show that acephate can cause damage to DNA. No further DNA repair tests are required.

3. Chromosomal effects.

A mouse micronucleus test was reported to be negative (Kirkhart, 1980, MRID GS0042-008). However, the test was not as sensitive as it should have been (not enough slides were examined) and was classified as acceptable only as a supplement to other tests. This test is not sufficient to satisfy the requirement for mutagenicity chromosomal effects testing, especially in view of the positive DNA repair assays. An *in vitro* chromosomal aberration analysis in mammalian cells in culture or an *in vivo* mammalian bone marrow chromosomal analysis should be performed. If these are positive, a heritable translocation test is required.

Metabolism (163.85-1)

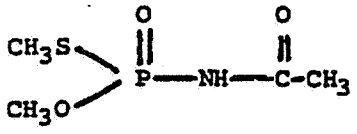
The minimum data requirement for metabolism is a single dose using the analytically pure grade of the active ingredient in the radioactively labeled form.

Studies with radioactive and nonradioactive acephate (Orthene) have shown that acephate is excreted essentially unchanged in mammalian urine. The excretion is rapid and there is no accumulation of acephate in tissues. About 0.6-1.6% of the administered acephate can be converted to methamidophos (Monitor) by the intestinal microorganisms of the rat and can, therefore, be absorbed along with the intact acephate. However, most of that methamidophos is rapidly eliminated from the animals.

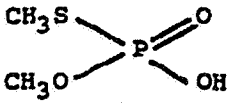
Lee (1972, MRID 00014994) studied the metabolism of Orthene in male and female rats, using S-methyl-$^{14}$C-Orthene. Orthene was rapidly and completely

absorbed from the stomach and was rapidly excreted in urine. About 87% and 95% of the administered activity was excreted, respectively, during the first 6 and 12 hours after dosing. Most of the remaining activity was found in the exhaled air (in $CO_2$; 1.0-4.5%), feces (1.0%), and tissues (0.4%).

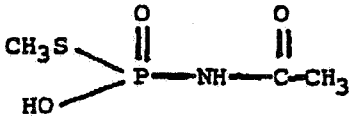
The activity found in urine was an unchanged Orthene (O,S-dimethyl acetylphosphoramidothioate; 73-77%)*, O,S-dimethyl phosphorothioate (3-6%)**, and S-methyl acetylphosphoramidothioate (3-4%)***. Methamidophos (O,S-dimethyl phosphoramidothioate)**** was not detected in urine. Male and female rats had the same excretion pattern.



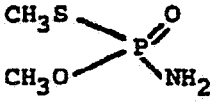
* O,S-dimethyl acetylphosphoramidothioate



**O,S-dimethyl phosphorothioate



*** S-methyl acetylphosphoramidothioate



**** O,S-dimethyl phosphoramidothioate

This study is adequate.

Similar results were obtained in another study with male Sprague-Dawley rats (Cheng, 1974, MRID 00014518). This study showed that treatment of rats with single oral doses of S-methyl-$^{14}$C-Orthene did not result in the hepatic binding or conjugation of the test material. Analysis of liver tissue at 3 hours after exposure and urine collected for 24 hours after exposure, showed that most of $^{14}$C was a free, unchanged Orthene (acephate). This study is acceptable.

Mammals do not metabolize acephate to methamidophos, but a portion of the administered acephate can be converted to methamidophos by the intestinal microorganisms of the mammals, as was shown by Warnock (1973, MRID 00014219). In that study, 6-week old male and female Sprague-Dawley rats were sacrificed at 3 hours after they were intubated one to four times with Orthene (100 mg/kg of body weight). The conversion took place in the small intestine and methamidophos was then absorbed into the blood stream.

The whole carcasses contained 0.6-1.6% and the excreta (chiefly urine) 1.1-1.5% of the final dose of Orthene as Ortho 9006 (methamidophos). There was as tendency for Ortho 9006 to accumulate in blood, liver, muscle, fat, and heart. Concentrations of Ortho 9006 in these tissues varied from 0.2 ppm to 1.1 ppm. Highest concentrations of Ortho 9006 were found in kidneys (4.1-11.5 ppm), testes (2.4-3.9 ppm), and brain (2.1 - 2.5 ppm).

The excretion pattern of Orthene was similar to that observed by Cheng (1974, MRID 00014518). Orthene was rapidly absorbed and rapidly eliminated by the rats. The whole carcasses contained 12-48% and the gastrointestinal tract

3-14% of the final dose at 3 hours after dosing. The excreta (chiefly urine) contained 54-56% of the final dose at 6 hours after dosing. There was some tendency for Orthene to concentrate in the testes, but not in other tissues. The elimination of Orthene and Ortho 9006 from the animal body was essentially complete in 24 hours after dosing. This study is adequate.

Dairy cattle, like rats, excreted the ingested Orthene rapidly in urine and did not accumulate it in tissues. In the study by Tucker (1973, MRID 00015225), lactating cows received 3, 10, or 30 ppm of Orthene and 0.6, 2, or 6 ppm of Ortho 9006 (Monitor), respectively, in a gelatin capsule for 30 consecutive days. The cows were observed for 6 days after the dosing stopped. Orthene was rapidly eliminated from the body. On recovery day 1, urine contained about one-third to one-half of the administered daily dose, and no Orthene was detected on recovery day 4. Milk contained about 0.1-0.2% of the daily dose of Orthene. These levels remained constant during the test, but there was no Orthene in milk on recovery day 2.

Orthene levels in tissues followed a linear dose-response relationship, but there was no accumulation. Kidney, heart, and muscle, in that order, contained the highest levels of Orthene. None of the tissues contained Orthene on recovery day 6. This study is acceptable.

Orthene disappeared rapidly from the tissues of pigs once exposure ceased. In the study by Tucker (1973, MRID 00015226), crossbred pigs were fed 3, 10, or 30 ppm of Orthene and 0.6, 2, or 6 ppm of Ortho 9006, respectively, for 30 days and were observed for 6 days (recovery period).

There was a linear relationship between treatment levels and tissue residues. The highest levels of Orthene detected on the 27th test day in the 30/6 ppm group was 0.49 ppm. Kidneys (0.42 ppm), muscle (0.48 ppm), and heart (0.49 ppm) contained the highest levels of Orthene. Brain and liver contained 0.25 ppm and 0.23 ppm of Orthene, respectively. On the recovery day 1, only traces of Orthene (0.02-0.03 ppm) were detected in muscle, kidney, heart, and brain tissue. By recovery day 6 (2nd sampling time), none of the tissues contained Orthene residues. This study is acceptable.

One goat, dosed orally with 40 mg (20 ppm) of $^{14}$C-S-methyl Orthene for 7 consecutive days, also rapidly excreted Orthene (Crossley and Lee, 1972, MRID 00015222). During dosing, about 8% of the daily $^{14}$C was detected in the general metabolic pool (that is, incorporated into proteins, lipids, nucleic acids, etc.); 66% and 3% appeared in urine and feces, respectively; and 3% was found in tissues (liver, kidney, heart, brain, and adipose tissue). About 95% of the excreted activity was eliminated from the body within 24 hours after dosing. An additional 6% of the total dose was excreted, mainly in urine, during the 10 days of recovery. About 80% of the radioactivity in the urine was unchanged Orthene (acephate), and less than 10% was associated with O,S-dimethyl phosphorothioate. Upon withdrawal of dosing, Orthene levels in the urine, tissues, and the metabolic pool declined rapidly.

Orthene level in milk was 0.95 ppm during dosing and about 0.1 ppm during the recovery day 7. Methamidophos was not detected in milk, urine, or tissues.

Because these data were obtained from only one animal, they are acceptable as supplementary.

Chicken fat, kidneys, and liver contained no detectable acephate or methamidophos when 24-week old chickens were fed 3, 10, or 30 ppm of Technical Orthene for 92 consecutive days (Leary and Lee, 1972, MRID 00015228). Muscle tissue had trace residues of acephate (Orthene) and methamidophos after 92 days of dosing at the 10 and 30 ppm levels. These residues equalled to about 0.01 ppm of acephate and 0.007 ppm of methamidophos. Seven days after the last dose (first sampling time), no residue of either chemical could be detected. This study is acceptable.

The above information is sufficient to satisfy the data requirement.

Augmentation Topics

There are no minimum requirements for these topics.

In Vitro Inhibition of RBC and Brain Acetylcholinesterase Activities by Acephate, Technical Acephate, and Methamidophos, and Their Mixtures. (Chevron Chemical Company, 1979, MRID GS0042-013).

Acetylcholinesterase (AChE) activity was assayed in vitro after incubating varying concentrations ($10^{-8}$ to $10^{-2}$ M) of acephate (99.3% pure), technical acephate (93.6% pure), and methamidophos (99.6% pure) with brain homogenates or RBC of rat and monkey. Concentrations of acephate with 1% or 3% methamidophos and technical acephate with 2% methamidophos were also incubated with rat brain and RBC preparations. The following results were obtained:

Inhibition (%) of RBC Acetylcholinesterase in the Rat

| Test Material | Concentration (M) | | | | | | |
|---|---|---|---|---|---|---|---|
| | $10^{-8}$ | $10^{-7}$ | $10^{-6}$ | $10^{-5}$ | $10^{-4}$ | $10^{-3}$ | $10^{-2}$ |
| Acephate (99.3%) | *** | | | | | | 55 |
| Technical acephate (93.6%) | | | | | 7 | 93 | -- |
| Methamidophos (99.6%) | | 23 | 55 | 67 | 86 | 90 | -- |
| Acephate + 1% methamidophos* | | | | 25 | 50 | 79 | -- |
| Acephate + 3% methamidophos* | | | | 14 | 57 | 86 | -- |
| Technical acephate + 2% methamidophos** | | | | 19 | 46 | 87 | -- |

Inhibition (%) of Brain Acetylcholinesterase in the Rat

| Test Material | Concentration (M) | | | | | |
|---|---|---|---|---|---|---|
| | $10^{-8}$ | $10^{-7}$ | $10^{-6}$ | $10^{-5}$ | $10^{-4}$ | $10^{-3}$ |
| Acephate (99.3%) | *** | | | | | 30 |
| Technical acephate (93.6%) | | | | 7 | 22 | 83 |
| Methamidophos (99.6%) | 8 | 12 | 25 | 72 | 94 | 93 |
| Acephate + 1% methamidophos* | | | | 10 | 22 | 82 |
| Acephate + 3% methamidophos* | | | 9 | 16 | 60 | 94 |
| Technical acephate + 2% methamidophos** | | | 7 | 12 | 58 | 95 |

*Mixtures containing 93.6% of acephate and 1% or 3% of methamidophos. Analytical grade (99.3% pure) acephate was used in these mixtures.

**Technical acephate (93.6% pure) to which methamidophos (final concentration: 2%) was added.

***Blank spaces in both tables denote zero inhibition. Horizontal line: assay not performed.

The following observations are apparent from these data:

1. Analytical grade acephate (Orthene) was a very weak inhibitor of brain and RBC AChE activities.

2. Technical acephate was a more potent inhibitor of brain and RBC acetyl cholinesterases than the highly purified acephate.

3. Highly purified methamidophos (Monitor) was about 1000 times and 10,000 times more potent than highly purified acephate in depressing brain and RBC AChE activities, respectively. The RBC AChE activity was about 5 times more susceptible to the action of methamidophos than was the brain enzyme.

4. Highly purified acephate with 3% methamidophos added and technical acephate with 2% methamidophos added caused similar inhibitions of RBC and brain AChE activities.

5. Highly purified acephate with 1% methamidophos added and technical acephate produced similar inhibitions of RBC and brain AChE activities.

These data indicate that the presence of methamidophos alone as an impurity in technical acephate can account for essentially all of the latter's ability to inhibit RBC and brain AChE activities.

The results obtained with monkeys were similar to those obtained with rats, except that methamidophos was 10 times less potent in inhibiting RBC AChE activity in the monkeys than in the rats. This study is acceptable.

## BIBLIOGRAPHY: PART I

Citations Used in Developing the Standards

Bio/dynamics Inc. (1980) Recovery from cholinesterase inhibition following dietary exposure to Orthene in the rat: Project No. 80-2454. Submitted by Chevron Chemical Company (MRID: GS0042-012)

Bio/dynamics Inc. (1981) A lifetime oral toxicity/carcinogenicity study with Technical RE-12420 in rats: Project No. 78-2135. Submitted by Chevron Chemical Company (MRID: GS0042-011)

Bozo Research Center, Japan (1979) Studies on acute delayed neurotoxicity of Orthene. Submitted by Chevron Chemical Company (MRID: GS 0042-005)

Cavalli, R.D. (1970) Acute Oral Toxicity of Ortho RE 12,420: SOCO 127/III:39. (Unpublished study received Jun 21, 1972 under 239-EX-61; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-F) (MRID: 00014675)

Cavalli, R.D. (1970) Effect of RE 12,420 on the Acetylcholinesterase of Rats: SOCO 123/III:31. (Unpublished study received Jun 21, 1972 under 239-EX-61; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-I) (MRID: 00014678)

Cavalli, R.D. (1970) Acute Dermal Toxicity of RE 12,420: SOCO 134/III:35. (Unpublished study received Jun 21, 1972 under 239-EX-61; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-L) (MRID: 00014681)

Cavalli, R.D. (1970) The Acute Inhalation Toxicity of RE 12,420: SOCO 174/III:105. (Unpublished study received Jun 21, 1972 under 239-EX-61; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-0) (MRID: 00014684)

Cavalli, R.D. (1970) Effect of RE 12,420 Vapors on the Acetylcholinesterase of Rats: SOCO 176/III:134. (Unpublished study received Jun 21, 1972 under 239-EX-61; submitted by Chevron Chemical Co.; Richmond, Calif.; CDL:223505-P) (MRID: 00014685)

Cavalli, R.D. (1970) Effect of Repeated Doses of RE 12,420 on Cholinesterase Activity in Rats: SOCO 147/III:55 (2). (Unpublished study received Jun 21, 1972 under 239-EX-61; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-R) (MRID:00014687)

Cavalli, R.D. (1970) Skin Sensitization Potential of RE 12,420: SOCO 130/III:34. (Unpublished study received Jun 21, 1972 under 239-EX-61; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-V) (MRID: 00014691)

Cheng, H.M. (1974) Characterization of 14C in Liver and Urine from Rats Treated Orally with S-Methyl-14C-Orthene or S-Methyl-14C-Ortho 9006. (Unpublished study received Nov. 10, 1976, under 239-2418; submitted by Chevron Chemical Co., Richmond, Calif.; CDL: 095570-L) (MRID: 00014518)

Chevron Chemical Company (1972) Two-year chronic oral toxicity study in beagle dogs: Final report. IBT No. C8732 (MRID: GS0042-006)

Chevron Chemical Company (1977) The acute dermal toxicity of Orthene Technical: SOCAL 1110/29:57 (MRID:GS0042-007)

Chevron Chemical Company (1979) Characterization of the in vitro inhibition of rat and monkey red blood cell and brain acetylcholinesterases by acephate, technical acephate, and methamidophos and their mixtures: SOCAL 1628. (MRID: GS0042-013)

Coquet, B. (1972) Determination of the Lethal Dose 50% by Intraperitoneal Route in the Male Rat of Ortho 12420 Technical (85% Acephate). (Translation; unpublished study received Mar 27, 1973 under 3F1375; prepared by Chevron Chemical Co. S.A.F., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:093670-J) (MRID: 00014935)

Crossley, J.; Lee, H. (1972) The fate of Orthene in locating ruminants (goats) - Final report. Submitted by Chevron Chemical Company (MRID: 00015222)

Ebbens, K. (1971) Report to Chevron Chemical Company, Ortho Division: Skin Sensitization Test with Orthene Technical, SX-284 in Albino Guinea Pigs: IBT No. A192. (Unpublished study received Jun 21, 1972 under 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-W) (MRID: 00014692)

Evans, E.L.; Mitchell, A.D. (1980) An evaluation of the effect of acephate on sister chromatid exchange frequencies in cultured Chinese hamster ovary cells. Project No. LSU-7558. Prepared by SRI International.

Fletcher, D. (1972) Report to Chevron Chemical Company, Ortho Division: Toxicity, Reproduction and Residue Study with Orthene Technical, SX-331 in White Leghorn Chickens: IBT No. J334. (Unpublished study received Aug 7, 1972 under 239-2406; prepared by Inustrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:051141-A) (MRID: 00014830)

Garofalo, M. (1973) Report to Chevron Chemical Company: A Study of the Effects of Orthene and Monitor on Plasma and Erythrocyte Cholinesterase Activity in Human Subjects during Subacute Oral Administration: IBT No. 636-02498. (Unpublished study received Mar 27, 1973 under 3F1375; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:093672-K)(MRID:00015160)

Haley, S. (1971) Report to Chevron Chemical Company, Ortho Division: Teratogenic Study with Orthene Technical in Albino Rats: IBT No. B190. (Unpublished study received Jun 21, 1972 under 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., Richmond, Calif.; CDL:223505-Z) (MRID: 00014695)

Hartke, K. (1971) Twelve-Month Status Report to Chevron Chemical Company, Ortho Division: Two-Year Chronic Oral Toxicity Study with RE 12,420 in Beagle Dogs: IBT No. C8732. (Unpublished study received Jun 21, 1972 under 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-AD) (MRID: 00014699)

International Research and Development Corp. (1979) Lifetime Oral carcinogenicity study in mice (1-year interim report); Project No. 415-006. Submitted by Chevron Chemical Company (MRID: GS0042-003)

International Research and Development Corp. (1981) Interim report of Technical (RE-12420) Orthene lifetime oral carcinogenicity study in mice. Histopathology data: Project No. 415-006. Submitted by Chevron Chemical Company (MRID: GS0042-002)

International Research and Development Corp. (1980) Teratology study in rabbits: Study No. 245262. Submitted by Chevron Chemical Company (MRID: GS0042-004)

Kirkhart, B. (1980) Micronucleus test on acephate. Project No. LSU-7558-19. Prepared by SRI International.

Kobayashi, S. (1978) Noyaku no dokusei ni okeru sojosayo (2) Shutoshite ko ChE satchuzai, 2-shu kongo no mausu kyusei keiko dokusei ni okeru sojosayo no kento. Synergism in pesticide toxicity: 2. Acute oral toxicity of anti-ChE pesticides in mice. Toho Igakkai Zasshi. Journal of Medical Society of Toho University. 25(4):635-649. (MRID: 05019361)

Kretchmar, B. (1972) Report to Chevron Chemical Company, Ortho Division: Acute Oral Potentiation Study with Monitor Technical and Orthene Technical in Albino Rats: IBT No A1117. (Unpublished study received Mar 27, 1973 under 3F1375; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:093670-I) (MRID: 0014934)

Ladd, R. (1972) Report to Chevron Chemical Company, Ortho Division: 30-Day Residue Study with Acephate/Ortho 9006 Mixture in Crossbred Pigs; IBT No. J2493. (Unpublished study received Mar 27, 1973 under 239-EX-60; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223489-I) (MRID: 00015227)

Leary, J.B.; Lee, H. (1972) Orthene--Chicken Feeding Test. (Unpublished study received Mar 27, 1973 Under 239-EX-60; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223489-J) (MRID:00015228)

Lee, H. (1972) Metabolism of Orthene in Rats. (Unpublished study including letter dated Jan 6, 1972 from C.F. Ott to J.N. Ospensen, received Feb 23, 1972 under 2G1248; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:091774-AC) (MRID: 00014994)

Levy, J.E.; Wong, Z.A. (1979) The Skin Irritation Potential of Orthene Specialty Concentrate: SOCAL 1418/39:12. (Unpublished study received Oct 31, 1979 under 239-EX-92; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:241253-E) (MRID: 00015305)

Levy, J.E.; Wong, Z.A. (1979) The Eye Irritation Potential of Orthene Specialty Concentrate: SOCAL 1419/39:10. (Unpublished study received Oct 31, 1979 under 239-EX-92; submitted by Chevron Chemical Co. Richmond, Calif.; CDL:241253-F) (MRID:00015306)

Mortelmans, K.E.; Riccio, E.S.; Shepherd, G.F. (1980) In Vitro Detection of Mitotic Crossing-Over, Mitotic Gene Conversion, and Reverse Mutation with S. Cerevisiae D7 for Seven Pesticides. Project No. LSU-7558-20. Prepared by SRI International.

Narcisse, J.K.; Cavalli, R.D. (1971) Eye Irritation Potential of Orthene Technical, Orthene 75S (CC-2153), and Orthene 75S (CC-2152): SOCAL 273/VI:107. (Unpublished study received Jun 21, 1972 under 239-EX-61; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-0) (MRID: 00014686)

Plank, J.B. (1972) Report to Chevron Chemical Company, Ortho Division: 90-Day Cholinesterase Study with Ortho 12,420 Technical in Albino Rats: IBT No. B9526. (Unpublished study received Aug 7, 1972 under 239-2406; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:051141-Q) (MDRID:00014832)

Rittenhouse, J.R. (1979) S-1458: The Acute Oral Toxicity of Orthene Specialty Concentrate: SOCAL 1416/37:80. (Unpublished study received Mar 11, 1980 under 239-EX-95; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:242078-C) (MRID:00029696)

Rittenhouse, J.R.; Narcisse, J.K.; Cavalli, R.D. (1972) The Acute Oral Toxicity to Rats of Orthene in Combination with Five Other Cholinesterase-Inhibiting Materials: SOCAL 383/XIV;41. (Unpublished study received Mar 27, 1973 under 3F1375; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:093670-H)(MRID:00014933)

Rittenhouse, J.R.; Wong, Z.A. (1979) The Acute Inhalation Toxicity of Orthene Specialty Concentrate: SOCAL 1420/36:104. (Unpublished study received Oct 31, 1979 under 239-EX-92; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:241253-G) (MRID:00015307)

Simmon, V.F. (1979 In vitro Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Eighteen Pesticides: Report No. EPA-600/1-79-041. (Unpublished study including submitter summary, received Apr 3, 1980 under 279-2712; prepared by SRI International, submitted by FMC Corp., Philadelphia, Pa.; CDL:099350-A) (MRID:00028625)

Tucker, B.V. (1973) Orthene and Ortho 9006 30 Day Pig Feeding Test--Residue Analysis of Tissues. (Unpublished study received Mar 27, 1973 under 239-EX-60; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223489-H) (MRID:00015226)

Tucker, B.V. (1973) Meat and milk residue study with Orthene and Ortho 9006 in dairy cattle. Submitted by Chevron Chemical Company (MRID:00015225)

Warnock, R.E. (1973) Metabolism of Orthene to Ortho 9006 Detected in Rats. (Unpublished study received Feb 17, 1977 under 6F1680; submitted by Chevron Chemical Co., Richmond, Calif.; CDL:098473-C) (MRID:00014219)

## BIBLIOGRAPHY: PART II

Citations Examined and Judged Inappropriate for Use in Developing the Standards

Arnold, D. (1971) Report to Chevron Chemical Company: Mutagenic Study with Orthene in Albino Mice: IBT No. E193. (Unpublished study received Jun 21, 1972 under 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-AB) (MRID:00014697)

Fletcher, D. (1972) Report to Chevron Chemical Company, Ortho Division: Neurotoxicity Study with Orthene Technical, SX-284 in Adult Hens: IBT No. J513. (Unpublished study received Jun 21, 1972 under 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-AC) (MRID:00014698)

Haley, S. (1972) Report to Chevron Chemical Company, Ortho Division: Three-Generation Reproduction Study with RE 12,420 Technical in Albino Rats: IBT No. B9272. (Unpublished study received Sep 2, 1972 under 2G1248; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:091772-A) (MRID:00014490)

Haley, S. (1973) Final Report to Chevron Chemical Company, Ortho Division: Three-Generation Reproduction Study with RE 12,420 Technical in Albino Rats: IBT No. B9272. (Unpublished study received Mar 27, 1973 under 3F1375; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:093670-AG (MRID:00014937)

Kretchmar, B. (1971) Report to Chevron Chemical Company, Ortho Division: Acute Oral Toxicity Study to Determine the Efficacy of Atropine sulfate and 2-PAM as Antidotes for RE 12,420 Technical Intoxication in Albino Rats: IBT No. A9376. (Unpublished study received Jun 21, 1972 under 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-J) (MRID:00014679)

Ladd, R. (1972) Report to Chevron Chemical Company, Ortho Division: Teratogenic Study with Orthene Technical in Albino Rabbits: IBT No. J191. (Unpublished study received Jun 21, 1972 uner 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-AA) (MRID:00014696)

Lindberg, D.C. (1971) Report to Chevron Chemical Company, Ortho Division: 90-Day Subacute Oral Toxicity Study with Orthene in Beagle Dogs: IBT No. C9527. (Unpublished study received Jun 21, 1972 under 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-T) (MRID:00014689)

Mastalski, K. (1970) Report to Chevron Chemical Company: Acute Oral Toxicity Study on RE 12,420 Technical in Beagle Dogs: IBT No. J8888. (Unpublished study received Jun 21, 1972 under 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-H) (MRID:00014677)

Mastri, C. (1970) Report to Chevron Chemical Company, Ortho Division: Acute Oral Toxicity Study on RE 12,420 Technical in White Mice: IBT No. A8889. (Unpublished study received Jun 21, 1972 under 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-G) (MRID:00014676)

Plank, J.B. (1971) Report to Chevron Chemical Company, Ortho Division: 90-Day Subacute Oral Toxicity Study with RE 12,420 Technical in Albino Rats: IBT No. B8887. (Unpublished study received Jun 21, 1972 under 239-EX-61; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:223505-S) (MRID:00014688)

Plank, J.B. (1973) Report to Chevron Chemical Company, Ortho Division: Two-Year Chronic Oral Toxicity Study with RE 12,420 Technical in Albino Rats: IBT No. B8733. (Unpublished study received on unknown date under 239-2406; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:051141-AB) (MRID:00014833)

Reyna, M.S. (1973) Report to Chevron Chemical Company, Ortho Division: 90-Day Subacute Oral Cholinesterase Study with Orthene Technical and Monitor Technical in Female Albino Rats: IBT No. B2442. (Unpublished study received Sep 5, 1972 under 239-2404; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:240996-A) (MRID:00014223)

61

Reyna, M.S. (1973) Report to Chevron Chemical Company, Ortho Division: 18-Month Carcinogenic Study with RE 12,420 Technical in Swiss White Mice: IBT No. B9269. (Unpublished study received Mar 27, 1973 under 3F1375; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:093671-D) (MRID:00014939)

Smith, P.S. (1972) Status Report to Chevron Chemical Company, Ortho Division: Two-Year Chronic Oral Toxicity Study with RE 12,420 Technical (SX-257) in Albino Rats: IBT No. B8733. (Unpublished study received Aug 7, 1972 under 239-2406; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:051142-C) (MRID:00014509)

Smith, P.S. (1972) Status Report to Chevron Chemical Company, Ortho Division: 18-Month Carcinogenic Study with RE 12,420 Technical (SX-257) in Swiss White Mice: IBT No. B9269. (Unpublished study received Aug 7, 1972 under 239-2406; prepared by Industrial Bio-Test Laboratories, Inc., submitted by Chevron Chemical Co., Richmond, Calif.; CDL:051142-D) (MRID:00014510)