CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
DICROTOPHOS

Chemical Code # 72, Document Processing Number (DPN) # 299
SB 950 # 60
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DATA GAP STATUS

Chronic toxicity, rat: No data gap, possible adverse effect
Chronic toxicity, dog: No data gap, possible adverse effect
Oncogenicity, rat: No data gap, no adverse effect
Oncogenicity, mouse: No data gap, possible adverse effect
Reproduction, rat: No data gap, possible adverse effect
Developmental toxicity, rat: No data gap, no adverse effect
Developmental toxicity, rabbit: No data gap, no adverse effect
Reverse mutation assay: No data gap, no adverse effect
In vitro mammalian cell assay: No data gap, possible adverse effect

In vivo cytogenetics assay: No data gap, no adverse effect

Neurotoxicity: No data gap, possible adverse effect †
† Hen neurotoxicity study did not indicate distal delayed neuropathies, but acetylcholinesterase inhibition was flagged as “possible adverse effect” in several rat studies.

Toxicology one-liners are attached.

All record numbers for the above study types through 280963 (Document No. 299-0069) were examined. This includes all relevant studies indexed by DPR as of Dec. 2, 2014.

In the 1-liners below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
## indicates a study on file but not yet reviewed.
NOTE: The following symbols may be used in the Table of Contents which follows:
** = data adequately address FIFRA requirement
† = study(ies) flagged as “possible adverse effect”
N/A = study type not currently required

This record contains summaries of studies. Individual worksheets may be useful for detailed assessment.

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METABOLISM AND PHARMACOKINETICS **
299-0053; 276578; “Dicrotophos: Rat Metabolism Study”; (D. Wu, Z. Gu; XenoBiotic Laboratories, Inc., Plainsboro, NJ; XLB Study No. XBL94040; 2/8/96); Five or seven Crl:CD(SD)/sex/group were assigned to one of 4 groups (designated A to D) and were treated with [3-14C] Dicrotophos (lot no. 836A-893, radiopurity: 98.9%, specific activity: 25.1 mCi/mmole). Non-labeled dicrotophos technical (lot no. DPAQ281, 96.0% E (cis) isomer, 1.45% Z (trans) isomer) was used to adjust the specific activity of the dosing preparations or as the dosing preparation in the multiple dose regimen. In Groups A, B and C, the rats were dosed orally by gavage. In Group D, they were injected intravenously with the test material. The Group A animals received a single dose of 0.5 mg/kg. The animals in Group B received 14 daily doses of 0.5 mg/kg of unlabeled dicrotophos and on the 15th day, a single dose of 0.5 mg/kg of the radiolabeled test material. In Group C, the animals received a single dose of 3.0 mg/kg. The Group D animals were dosed once with 0.5 mg/kg. The primary route of excretion was via the urine with the percentage of administered dose recovered from the urine ranging from 86 to 89 (urine and cage rinse) by the conclusion of the 4-day collection period irrespective of the dosing regimen. Recovery in the feces ranged from 1.5 to 5% of the administered dose. Ninety one to 95% of the administered dose was excreted within the 1st 24 hours. These data indicated that approximately 94 to 97% of the administered dose was absorbed. Analysis of the tissues at 4 days post dose or post-final dose revealed the primary site of radiolabel recovery to be the liver. In the metabolite analysis, the parent compound constituted 3 to 7% of the administered dose. The formation of monocrotophos by demethylation of one of the amide methyl groups was <1 to 3% of the dose. Cleavage of the phosphate group with the resultant formation of the acetooacetamide moiety and subsequent hydroxylation of the methyl groups and/or reduction of one of the carbonyl oxygens was the primary pathway of metabolism. Study Acceptable. (Moore, 8/28/14)
GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

Acute oral toxicity, rat ** † (flagged because Toxicity Category I)

299-0018; 45362; “Toxicology of Insecticides: The Acute Oral and Percutaneous Toxicity, Skin and Eye Irritancy and Skin Sensitizing Potential of Bidrin”; (J.B. Price; Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent, ME9, 8AG, England; Doc. No. SBGR.85.266; 12/12/85); Five Fischer 344 rats/sex/group were dosed orally by gavage with 5, 8, 12, 20 or 30 mg/kg of Bidrin (Dicrotophos technical) (batch no. 17-1-0-0; Dicrotoph os-E content: 88.3%) (vehicle: water). The following mortality resulted from the treatment: 5 (M/F: 0/5), 8 (M: 0/5, F: 2/5), 12 (M: 4/5, F: 5/5), 20 (M/F: 5/5), 30 (M/F: 5/5). Deaths occurred within 90 minutes of dosing. Clinical signs included lacrimation, salivation, fasciculation, chromodacryorrhea, unkempt appearance, and abnormal posture. In the necropsy examination, those animals which died prematurely had discolored liquid in the stomachs and minor hemorrhages in the cranial cavity or brain surface. **Rat Oral LD50:** (M) 11 mg/kg; (F) 8 mg/kg; Toxicity Category I; **Study acceptable.** (Kahn, 3/21/86, updated Moore, 1/24/14)

Acute dermal toxicity **

299-0018; 45363; “Toxicology of Insecticides: The Acute Oral and Percutaneous Toxicity, Skin and Eye Irritancy and Skin Sensitizing Potential of Bidrin”; (J.B. Price; Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent, ME9, 8AG, England; Doc. No. SBGR.85.266; 12/12/85); The skin of five Fischer 344 rats/sex/group (except where noted) was exposed to 80, 125, 200, 315 (10 animals/sex), 500 (10 animals/sex), 800 or 1270 mg/kg of Bidrin (Dicrotophos technical) (batch no. 17-1-0-0; Dicrotoph os-E content: 88.3%) for 24 hours under an occlusive wrap. Water was used to dilute all of the treatment preparations except for the 1270 mg/kg treatment which was undiluted. The following mortality resulted from the treatment: 80 (M/F: 0/5), 125 (M/F: 0/5), 200 (M/F: 0/5), 315 (M: 0/9 (one animal escaped during the observation period), F: 3/10), 500 (M: 0/10, F: 3/10), 800 (M: 1/5, F: 5/5), 1270 (M/F: 5/5). Clinical signs included fasciculations, chromodacryorrhea, tremors, hunched back, lethargy and unkempt appearance. Some survivors demonstrated body weight loss over the 14-day observation period. In the necropsy examination for those animals dying prematurely, gastrointestinal tract abnormalities, intracranial hemorrhages and prominent subcutaneous blood vessels at the application site were noted. **Rat Acute Dermal LD50:** (M) 876 mg/kg, (F) 487 mg/kg; Toxicity Category II; **Study acceptable.** (Kahn, 3/21/86, updated Moore, 1/27/14)

Acute inhalation toxicity, rat **

0037, 276007; “Dicrotophos: 4-Hour Acute Inhalation Toxicity Study in Rats” (Noakes, J.P., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Report No. CTL/HR2512/Regulatory/Report, Study No. HR2512, 11/08/2004). 870.1300. Dicrotophos (technical material) (Lot # 403001B, purity = E isomer 87.2%, Z isomer 2.8%) was aerosolized and administered in a nose-only manner under dynamic conditions to 5 Alpk:APfSD rats per sex per dose at a dose level (mean gravimetric concentration) of 0.061 mg/l (with a mean MMAD (GSD) of 2.72 (4.00) um) for 4 hours. No mortalities occurred during exposure or during the 14-day observation period. Decreased activity, increased breathing depth, reduced breathing rate, irregular breathing, chromodacryorrhea, reduced foot withdrawal reflex, abnormal respiratory noise, salivation, reduced response to sound, shaking, staining around the nose, and wet fur were observed in both sexes after exposure; hunched posture and increased response to touch were also observed in the females. All of these clinical signs resolved by day 2 except for increased breathing depth and increased response to touch in females which resolved by day 3 and day 5,
respectively. Necropsy revealed no macroscopic abnormalities. LC$_{50}$ (M/F) > 0.061 mg/l. Toxicity Category II. Acceptable. (Corlett, 03/06/2014)

**Primary eye irritation, rabbit**

299-0018; 45365; “Toxicology of Insecticides: The Acute Oral and Percutaneous Toxicity, Skin and Eye Irritancy and Skin Sensitizing Potential of Bidrin”; (J.B. Price; Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent, ME9, 8AG, England; Doc. No. SBGR.85.266; 12/12/85); The eyes of 6 New Zealand White rabbits were treated by conjunctival instillation with 0.1 ml/eye of Bidrin (Dicrotophos technical) (batch no. 17-1-0-0; Dicrotophos-E content: 88.3%). There was no corneal opacity noted throughout the 14-day observation period. Iritis, grade 0.5 (1/6), was evident at 24 hours post-dose, clearing by 48 hours. Conjunctival redness, grades 2 (3/6) and 1.5 (3/6), were noted at 24 hours post-dose, diminishing to grades 1 (1/6) and 0.5 (4/6) at 7 days, clearing by 14 days. Chemosis, grade 1 (4/6), was evident at 24 hours, clearing by 7 days. Discharge, grade 0.5 (6/6), was noted at 24 hours, clearing by 48 hours. Within 1 hour of dosing, the animals demonstrated constricted pupils and were lying prone, recovering approximately 2.5 hours after dosing. Toxicity Category III; Study acceptable. (Kahn, 3/21/86, updated, Moore, 1/27/14)

**Primary dermal irritation**

299-0018; 45364; “Toxicology of Insecticides: The Acute Oral and Percutaneous Toxicity, Skin and Eye Irritancy and Skin Sensitizing Potential of Bidrin”; (J.B. Price; Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent, ME9, 8AG, England; Doc. No. SBGR.85.266; 12/12/85); The skin of 6 New Zealand White rabbits was exposed to 0.5 ml/site, one site/animal of Bidrin (Dicrotophos technical) (batch no. 17-1-0-0; Dicrotophos-E content: 88.3%) for 4 hours under a semi-occlusive patch. No erythema or edema were noted throughout the 7-day observation period. Toxicity Category IV; Study acceptable. (Kahn, 3/21/86, updated, Moore, 1/27/14).

**Dermal sensitization**

299-0018; 45366; “Toxicology of Insecticides: The Acute Oral and Percutaneous Toxicity, Skin and Eye Irritancy and Skin Sensitizing Potential of Bidrin”; (J.B. Price; Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, Kent, ME9, 8AG, England; Doc. No. SBGR.85.266; 12/12/85); Twenty Dunkin-Hartley guinea pigs received a total of 6 intradermal injections of 0.1 ml each, 2 each of Freund’s Complete Adjuvant: distilled water (1:1), 0.5% (w/v) dilution of Bidrin (Dicrotophos technical) (batch no. 17-1-0-0; Dicrotophos-E content: 88.3%) in water, and a 0.5% dilution of the test material in a 50:50 mixture of Freund’s Complete Adjuvant and water on day 0 of induction. On day 7, the skin of the treated animals was exposed to a filter paper saturated with 0.3 ml of the undiluted test material for 48 hours under an occlusive wrap as the second induction treatment. Ten control animals were treated in the same manner except that the test material was not included in the dosing regimen. Two weeks after the topical induction application, the skin of each of the animals was exposed to a filter paper saturated with 0.1 ml of the undiluted test material for 24 hours under an occlusive wrap. In the challenge, thirteen of the 20 induced animals demonstrated a positive response at 24 hours post-exposure, diminishing to 12 animals at 48 hours. No response was noted for the control animals. The test material is a dermal sensitizer in accordance with the Guinea Pig Maximization Test. The positive control was functional. Study acceptable. (Kahn, 3/21/86, updated, Moore, 1/27/14)
SUBCHRONIC STUDIES

Oral toxicity, rat: (No standard rat subchronic, but see 90-day neurotoxicity, below)
Study not submitted.

Oral toxicity, non-rodent:
Study not submitted.

Dermal toxicity, 21/28-day or 90-day: ** † (flagged for brain AChE inhibition)
**299-0060; 280092; “Dicrotophos: 21/28 Day Dermal Toxicity Study in the Rat”; (J.P. Noakes; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ; Study No. LR0588; 2/14/01); The skin of 15 Crl:CD rats/sex/group was treated with 0 (deionized water), 2, 5, 10 or 80 mg/kg/day of Dicrotophos technical (batch no. 403001B; purity: 87.6%) for 6 hours/day for 21 days over a 28-day period. Five of the animals/sex/group were identified as a satellite cohort in which cholinesterase activity was assayed in the brain, red blood cell and plasma at the conclusion of the treatment period. One control female, one female in the 2 mg/kg group, one male in the 5 mg/kg group and one male and four females in the 80 mg/kg group were found dead between study 18 and 21. The report author did not attribute the deaths to treatment because 7 of the 8 deaths occurred at a time when the animals had not been dosed but had been bandaged. The deaths were attributed to poor bandaging. In the clinical observations, an increasing incidence of erythema was noted for the females in all of the treatment groups in a dose-related manner. This effect was not noted for the males. There was no treatment-related effect upon the mean body weights of the animals in the main study. However, the males in the 80 mg/kg treatment of the satellite cohort demonstrated lower mean body weights over the course of the study. There was no treatment-related effect upon food consumption of the main study group. Ophthalmological examination did not reveal any treatment-related effects. There were no treatment-related effects noted in the FOB and motor activity assessment. None of the hematology parameters were affected by the treatment. No treatment-related effects were evident in the clinical chemistry assessment. Cholinesterase activity was reduced in the brains of both sexes in the 10 and 80 mg/kg treatment groups (>25% reduction) (p<0.01 or 0.05). Similar decrements in red blood cell and plasma activity levels were noted as well. The absolute and/or relative organ weights were not affected by the treatment. There were no treatment-related lesions noted in the histopathological examination. Possible adverse effect: significant reduction in brain cholinesterase activity. Rat 21/28 Day Repeated Dosing Dermal Toxicity NOEL: (M/F) 5 mg/kg/day (based upon the significant reduction in brain cholinesterase activity noted in both sexes of the 10 mg/kg treatment group); Study acceptable. (Moore, 9/19/14) not submitted.

**299-0061; 280093 This is an exact duplicate of 299-0060; 280092, above.

Inhalation toxicity, 28-day to 90-day: (N/A) † (flagged for brain AChE inhibition)

299-0040; 276563; “Dicrotophos Technical: Toxicity Study by Snout-Only Inhalation Administration to CD Rats for 4 Weeks”; (J. A. Blair; Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID No. BDG0002; 6/17/10); Ten Crl:CD (SD) rats/sex/group were exposed nose-only to 0, 0.097, 0.73, or 2.9 µg/l
(analytical) of Dicrotophos technical (batch no. GB101309-01; purity: 88.9%) for 6 hours/day, 5 days/week for 4 weeks. The exposure atmosphere consisted of both a particulate and a vapor phase with 90 to 99% of the test material was either vapor or a particle size less than 7 µm. No deaths occurred during the study. The mean body weights and food consumption were not affected by the treatment. In the hematological evaluation, the mean percentage of reticulocytes was reduced for the males in the 2.9 µg/l exposure group (p<0.05). No apparent treatment-related effects were noted for the clinical chemistry parameters. Red blood cell cholinesterase (AChE) activity was reduced for both sexes in the 0.73 and 2.9 µg/l exposure groups (p<0.01). Brain AChE activity was reduced for both sexes in the 0.73 and 2.9 µg/l exposure groups and for the females in the 0.097 µg/l exposure group (p<0.05 or 0.01). There was no treatment-related effect upon the mean organ weights. Atrophy of the seminiferous tubules in the testes of males in the 2.9 µg/l exposure group was noted (0: 0/10 vs. 2.9: 2/10). Possible adverse effect: significant reduction in brain acetylcholinesterase activity; Rat 28-Day Inhalation Toxicity NOEL: (M) 0.097 µg/l (based upon significant reduction in AChE activity in the brain of the 0.73 µg/l exposure group; (F) < 0.097 µg/l (based upon the significant reduction in AChE activity in the brain of the 0.097 µg/l exposure group); Study supplemental (Non-guideline study). (Moore, 8/19/14)

**CHRONIC STUDIES**

Combined Chronic and Oncogenicity, rat **† (flagged for brain AChE inhibition)**

** 299-0028; 273372; “Dicrotophos: Two Year Dietary Toxicity and Oncogenicity Study in Rats”; (S.L. Allen; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. PR0986; 2/23/98); Fifty two Alpk:APfSD rats/sex/group received 0, 0.5, 5.0 or 25 ppm of Dicrotophos technical (batch no. 403001 B; purity: 87.65% (E isomer)) in the diet for up to 105 weeks ((M) 0, 0.02, 0.25, 1.42 mg/kg/day, (F) 0, 0.03, 0.32, 1.74 mg/kg/day). A satellite cohort of 12 animals/sex/group received the test material in the diet for up to 53 weeks. An additional 16 animals/sex/group were treated for up to 105 weeks and were utilized for the measurement of plasma, red blood cell and brain cholinesterase (ChE) activities at the termination of the study. Eight additional animals/sex/group were treated for 53 weeks and plasma, red blood cell and brain ChE activities were measured at that time. The males in the 25 ppm group demonstrated aggressive behavior, irregular breathing, involuntary shaking of the limbs, urine staining, and hunched posture. The females in the 25 ppm group demonstrated an increased incidence of irregular breathing, involuntary shaking of the limbs, hunched posture, abnormal respiratory noise and piloerection. The females in both the 5 and 25 ppm groups exhibited an increased incidence of urine staining. The survival of the 25 ppm males was so affected by the treatment that surviving animals in that group were euthanized during weeks 95 to 97. The males in the 5.0 ppm group also demonstrated reduced survival such that the remaining groups were euthanized during weeks 99 and 100. The number of females in the 25 ppm group which survived to week 105 was only 29% as well. The mean body weight of the 25 ppm males was lower than that of the control group throughout the study. The 25 ppm females experienced a lower mean body weight in comparison to the controls during the first weeks of the study, recovering thereafter. The mean food consumption for both sexes in the 25 ppm group was less than that of the control group during the first month of the study. Thereafter, food consumption did not appear to be affected by the treatment. Although certain of the
hematological and clinical chemical parameters demonstrated statistically significant differences between the 25 ppm and control groups, there was no consistent effect upon these parameters which exhibited a physiologically significant response. In the urinalysis there was a consistent reduction of the volume and increase in the specific gravity of the urine samples collected from both sexes in the 25 ppm group in comparison to the control group over the course of the study. In the necropsy examination, there was no treatment-related effect upon organ weights. Increased incidences of focal atrophy/degeneration of the acinar epithelium of the Harderian gland and aspiration pneumonia were noted for the females in the 25 ppm group. In the cholinesterase assay, significant reduction in brain, plasma and red blood cell ChE activities was noted for both sexes in the 0.5 ppm group. This result is pertinent because the activities of the latter two ChEs are monitored in the field in an effort to provide surveillance for agricultural workers. This monitoring effort is considered to be health protective because generally the activities of these two cholinesterases are reduced at a concentration which is much lower than the level at which brain cholinesterase is affected. However in this instance that is not the situation. The workers could possibly suffer significant reduction of brain ChE activity before their plasma and/or red blood cell ChE activity levels are sufficiently reduced to warrant the worker’s removal from the field; Possible adverse effect: significant reduction of brain ChE activity; Rat Chronic Dietary Toxicity NOEL: (M/F) < 0.5 ppm ((M) <0.02 mg/kg/day, (F) <0.03 mg/kg/day) (based upon the reduced brain cholinesterase activity of both sexes in the 0.5 ppm group); no oncogenicity was evident. Study acceptable. (Moore, 10/10/13)

299-016 036509 “Bidrin: Safety evaluation by a chronic feeding study in the rat for two years,” (final report), Howard, D. J., Donoso, J., and Johnston, C. D., Woodard Research Corporation, 9/21/1967. This study employed only 25 rats/sex in treated groups (40/sex in controls). Rats were of unknown strain obtained from Charles River Laboratories, maintained for up to 2 years. The study was hampered by respiratory disease. A high percentage of decedents had substantial autolysis of tissues. No increases in tumors were indicated. Given the availability of a contemporary acceptable combined rat chronic/oncogenicity study, there is no reason to pursue this older study further. Aldous, 11/26/14 (no DPR worksheet).

Chronic, dog ** † (flagged for reduced brain AChE activity)

299-0023, -0055; 273356, 276580; “Dicrotophos: 1-Year Oral Toxicity Study in Dogs”; (S.A. Horner; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ; Study No. PD1008; 6/27/97); Four beagle dogs/sex/group were scheduled to be dosed via capsule with 0, 0.025, 0.1 or 1.0 mg/kg/day of Dicrotophos technical (batch no. 403001 B; purity: 87.65%, dosing was adjusted for the purity of the test material) for one year. After 13 weeks, treatment of the high dose level was discontinued for a week, and then resumed at 0.5 mg/kg/day for the remainder of the study. All of the animals survived to the termination of the study. The mean body weights of the 0.1 and 0.5 mg/kg females were less than the control group by the termination of the study (p<0.05). There was no apparent treatment-related effect upon the food consumption of the treated animals. Clinical signs included salivation by the females in the 1.0/0.5 mg/kg treatment group. The females in both the 0.1 and 1.0/0.5 mg/kg groups also demonstrated a markedly increased incidence of salivation at the time of dosing. An increased incidence of fluid feces was noted for both sexes in the 1.0/0.5 mg/kg treatment group, particularly during the 1st 13 weeks when they were being treated with 1.0 mg/kg/day. Regurgitation was observed for both sexes in the 1.0/0.5 mg/kg group during week 13. The
hematology evaluation and urinalysis did not reveal any treatment-related effects. In the clinical chemistry evaluation, the serum albumin and calcium levels for both sexes in the 1.0/0.5 mg/kg treatment group were lower than the control values at various times during the study. The serum cholesterol level for the females in the high dose group was also less than that of the control group throughout the treatment period. The plasma cholinesterase (ChE) activities for both sexes in the 0.025 mg/kg treatment group and above were reduced in a treatment-related manner in comparison to the control group activity (p<0.01). The red blood cell ChE activities of both sexes in the 1.0/0.5 mg/kg group and the males in the 0.1 mg/kg group were less than that of the control group (p<0.05 or 0.01). The brain ChE activities of both sexes in the 1.0/0.5 mg/kg group and the females in the 0.1 mg/kg group were less than the control group value (p<0.05 or 0.01). In the necropsy examination, there was no treatment-related effect on the mean organ weights. The histopathological examination did not reveal any treatment-related lesions.

Possible adverse effect: significant reduction in brain ChE activity.

Dog Chronic Oral Toxicity NOEL: (M/F) <0.025 mg/kg/day (based upon the significant reduction in plasma cholinesterase activity for both sexes in the 0.025 mg/kg treatment group); Previously the study was unacceptable, possibly upgradeable with the submission detailing how the ophthalmological examination was performed; the information provided in record no. 276580 was sufficient to document that the ophthalmological examination was performed; Study acceptable. (Moore, 8/28/14)

299-016 036510 “Bidrin: Safety evaluation by a chronic feeding study in the dog for two years,” (final report), Johnston, C. D., Thompson, W. M., and Donoso, J.; Woodard Research Corporation, 9/28/1967. This older study involved 3 beagle dogs/sex/group at 0.16, 1.6, or 16 ppm dicrotophos for 2 years, or 2 dogs/sex at 100 ppm dicrotophos for one year. Investigators reported “fairly consistent salivation, soft stools, and/or tremors in the 100-ppm beagles,” with occasional instances of these findings at lower dose levels. Those results of the 100 ppm group may be of interest, because this dose was out of the range of levels used in the accepted study above. Given the availability of a more recent guideline chronic dog study, there is no reason to pursue results of this older study further. Aldous, 11/26/14 (no DPR worksheet).

Oncogenicity, rat (see Combined, above)

See Chronic Toxicity, rat above.

Oncogenicity, mouse ** † (flagged for thyroid adenomas)

** 299-0024; 273357; “Dicrotophos: Two Year Oncogenicity Study in Mice”; (G.M. Milburn; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. PM0992; 1/7/98); Fifty five C57BL/10J,CD-1 mice/sex/group received 0, 2, 10 or 50 ppm of Dicrotophos technical (batch no. 403001 B; purity: 87.65%) in the diet for up to 105 weeks ((M) 0, 0.22, 1.12, 6.42 mg/kg/day, (F) 0, 1.58, 9.06 mg/kg/day). The survival of the females in the 50 ppm group was reduced to such an extent that they were euthanized during week 101. The mean body weights of both sexes in the 50 ppm group were less than those of the control group during the first several months of treatment. Thereafter the effect was no longer evident. Food consumption for these animals was less than that of the control week during the first week of the study. No treatment-related effect was apparent thereafter. There were no apparent treatment-related effects noted in the ophthalmoscopic examination. The hematology evaluation did not
reveal any treatment-related effects on the differential white blood counts or the other hematological parameters. In the histopathological examination, there was a treatment-related increase in renal tubular vacuolation for the 50 ppm males in terms of incidence and severity of the lesion in comparison to the controls (0: 23/55 vs. 50: 39/55). The incidence of follicular cell adenoma was also noted in the thyroid glands of these animals (0: 0/54 vs. 50: 5/49). Possible adverse effect: follicular cell adenoma in the thyroid gland. Mouse Chronic Dietary NOEL: 10 ppm ((M) 1.12 mg/kg/day, (F) 1.58 mg/kg/day) (based upon the initial reduction in body weight of both sexes, the incidence of tubular vacuolation in the kidneys of the 50 ppm males and the reduced survival of the females in the 50 ppm group); oncogenicity: follicular cell adenomas in the thyroid gland. Study acceptable. (Moore, 9/25/13)

GENOTOXICITY

Bacterial Reverse Mutation Assay **
** 299-0030; 273375; “Salmonella Plate Incorporation Mutagenicity Assay (Ames Test) with a Confirmatory Assay”; (R.H.C. San, M.K. Wyman; Microbiological Associates, Inc., Bethesda and Rockville, MD; Study No. G94AW39.501001; 12/2/94); S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 were treated with Dicrotophos technical (batch no. 403001B; purity: 87.65%) at concentrations ranging from 100 to 5000 µg/plate under conditions of (+/-)-activation, using the plate incorporation method, for 48 to 72 hours at 37°C in two trials. Each treatment level was plated in triplicate. An Aroclor 1254-induced rat liver S9 fraction was used to metabolize the test material. There was no apparent treatment-related increase in the incidence of reverse mutations in any of the strains under conditions of (+/-)-activation. No adverse effect. The positive controls were functional. Study acceptable. (Moore, 10/16/13)

In Vitro Mammalian Cell Assay ** † (positive mouse lymphoma assay)
** 299-0030; 273376; “L5178Y/TK+/− Mouse Lymphoma Mutagenesis Assay with a Confirmatory Assay”; (R.H.C. San, J.J. Clarke; Microbiological Associates, Inc., Rockville, MD; Study No. G94AW39.702001; 1/16/95); Mouse lymphoma L5178Y cells (clone 3.7.2C (TK+/−)) were treated with Dicrotophos technical (batch no. 403001B; purity: 87.65%) at concentrations ranging from 100 to 3000 µg/ml under conditions of activation and non-activation for 4 hours at 37°C. Two independent trials were performed with 2 replicates per treatment. An Aroclor 1254-induced rat liver S9 fraction was used to activate the test material. Cell viability and mutation frequency were determined and compared to the solvent control level. There was a treatment-related increase in the mutation frequency above that of the solvent control under conditions of both activation and non-activation. Adverse effect indicated. The positive controls were functional. Study acceptable. (Moore, 10/17/13)

In Vivo Cytogenetics Assay **
11/15/94); Five ICR mice/sex/group/time point were dosed by intraperitoneal injection (ip) with 0 (distilled water), 1.7, 3.3, or 6.6 mg/kg of Dicrotophos technical (batch no. 403001B; purity: 87.65%). For the positive control, five mice/sex were dosed ip with 40 mg/kg of cyclophosphamide. Treated animals were euthanized at 24, 48 and 72 hours after dosing. The animals which were treated with the positive control were euthanized at 24 hours post dose. Femoral bone marrow was harvested and evaluated for the presence of micronuclei in polychromatic erythrocytes (PCE). One thousand polychromatic erythrocytes were evaluated per animal. One male and three females in the 6.6 mg/kg group died and were replaced. Treatment with the test material did not result in an increase in the number of micronuclei per 1000 PCE’s. No adverse effect indicated. The positive control was functional. Study acceptable. (Moore, 10/16/13)

299-017 036517 Dean, B. J. and K. Senner, “Chromosome studies on bone marrow cells of mice after a single oral dose of Bidrin,” Tunstall Laboratory, Dec. 1973. In a study which pre-dated current guidelines, and which had no QA oversight and no concurrent positive controls, Bidrin was administered to male and female mice at 0, 5, or 10 mg/kg at 8 hrs or 24 hrs prior to sacrifice and examination of bone marrow cells. There was no increase in chromatid gaps or breaks, and no effect on polyploidy associated with Bidrin treatment. No adverse effects are indicated. Supplementary data: no DPR worksheet. Aldous, 12/1/14.

Miscellaneous Genotoxicity Assays (not classifiable with current guidelines)

299-017 036515 Doak, S. and C. Whitebread, “Toxicity studies with Bidrin in the host-mediated assay and with microorganisms in vitro,” Tunstall Laboratory, July 1974. This brief (7-page) report describes direct (buffered solution) and host mediated (mouse ip injection of cells) exposures of a double auxotrophic strain of Saccharomyces cerevisiae to dicrotophos at a range of dose levels. The host-mediated trials were negative. Bidrin was weakly positive in some direct trials at 5 to 10 µg/ml, negative at 20 µg/ml, and clearly positive at 50 µg/ml (5% solution). Thus study indicates a “possible adverse effect,” although reliably so only at very high dose levels. This Saccharomyces cerevisiae test system is no longer commonly used. Since there is an accepted positive eukaryotic cell gene mutation assay already (Record No. 273376), and since this study pre-dates current guidelines, there is no worksheet for this report. Aldous, Dec. 1, 2014.

299-017 036518 Dean, B. J., “Dominant lethal in male mice after single or repeated oral dosing with Bidrin,” Tunstall Laboratory, Nov. 1974. Typically 12 male mice/group were dosed once with Bidrin at 5 or 10 mg/kg in Trial 1, or in Trial 2 either with a single dose of 10 mg/kg Bidrin, or with 1 or 2 mg/kg/day for 5 consecutive days. Twenty-four untreated controls were used in each trial, and MMS was used as a positive control in Trial 1 only. Bidrin did not cause consistent effects on percentage pregnancies in groups, or on total implants per pregnant female, or (most importantly) on early fetal deaths. This study pre-dates current guidelines. As this is a negative study, there is no DPR worksheet for this report. Aldous, Dec. 1, 2014.
**REPRODUCTIVE TOXICITY, RAT ** † (flagged for excessive pup mortality)

** 299-0029; 273373; “Dicrotophos: Multigeneration Study in the Rat”; (M.E. Moxon; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. RR0689; 3/18/97); In the F0 generation, twenty six Wistar rats/sex/group were scheduled to receive 0, 0.5, 5.0 or 25 ppm of Dicrotophos technical (batch no. 403001 B; purity: 87.65% (E isomer)) in the diet for 10 weeks of pre-mating, during mating, and 3 weeks of gestation and 4 weeks of lactation. Due to the high loss of offspring in the 25 ppm group, the treatment was reduced to 10 ppm from lactation day 8 through 29. A second mating (designated F1B) of the F0 generation was instituted in which the parents in the high dose group were treated with 15 ppm of the test material from mating through the end of the lactation period. At the time of the selection of the F1 adults from the F1B litters, the concentration was readjusted to 10 ppm for the remainder of their treatment (i.e., 10-week pre-mating, mating, gestation and lactation periods) ((M) 0, (0.5 ppm) 0.05, (5.0 ppm) 0.49 to 0.56, (25 ppm) 2.53, (10 ppm) 1.15 mg/kg/day, (F) (0.5 ppm) premating: 0.05 to 0.06, gestation: 0.04, lactation: 0.11 to 0.12 mg/kg/day, (5.0 ppm) premating: 0.53 to 0.59, gestation: 0.42 to 0.44, lactation: 1.02 to 1.15 mg/kg/day, (25 ppm) premating: 2.79 mg/kg/day, (10 ppm) premating: 1.25, gestation: 0.89, lactation: 2.08 mg/kg/day, (15 ppm) gestation: 1.29, lactation: 2.46 mg/kg/day). There was no apparent effect upon the survival of the parental generations. Involuntary shaking of the limbs was noted for both sexes in the 25 ppm treatment group (F0 generation) during the first weeks of the pre-mating period. The mean body weights of the adults in the 5.0 ppm and above treatment levels were less than the control body weights during the pre-mating and lactation time period (NS, p<0.05 or 0.01). The mean body weights during the gestation periods of both generations were not affected by the treatment. The mean food consumption of both sexes in the 25 ppm treatment group was less than that of the control group in the F0 generation during the 1st month of the pre-mating period. Thereafter there was no treatment-related reduction on food consumption until the lactation periods of the F0 generation (10 and 15 ppm treatment groups) and the lactation period of the F1 generation (5.0 and 10 ppm treatment groups). The fertility indices of the dams in the high dose group of the F0 generation (25 and 15 ppm treatment levels) were lower than that of the control group. At a treatment level of 10 ppm for the F1 generation, no effect on fertility was evident. The gestation indices were not affected at any of the treatment levels. Pup viability indices were affected in a treatment-related manner at the 5 ppm treatment level and above for both generations. There was no apparent treatment-related effect upon the pup weights. Possible adverse effect: excessive pup mortality; Parental NOEL: 0.5 ppm ((M) 0.05 mg/kg/day; (F) 0.05 to 0.06 mg/kg/day) (based upon treatment-related effect upon the body weights of both sexes in the 5.0 ppm treatment group); Reproductive NOEL: 10 ppm (1.25 mg/kg/day) (based upon the reduced fertility indices for the 15 ppm treatment group and above); Developmental NOEL: 0.5 ppm (0.05 to 0.06 mg/kg/day) (based upon the reduced pup viability noted for the 5.0 ppm treatment groups of both generations); Study acceptable. (Moore, 10/15/13)

299-017 036514 “Results of reproduction study of rats fed diets containing Bidrin insecticide over three generations,” Eisenlord, G., The Hine Laboratories, Aug. 1965. This 14-page report describes a study in which rats were initially administered 0, 2, 5, 15, or 50 ppm Bidrin. The 50 ppm dose group was discontinued after F1b littering period due to weakness and weight loss in parents, CNS signs such as tremors and incoordination in pups, and high mortality in litters. This study pre-dates current guidelines, and cannot be made acceptable, and is designated as
supplementary data. There is no DPR worksheet, since the accepted study above (Record No. 273373) spanned an effective dose-response range. Aldous, Dec. 1, 2014.

DEVELOPMENTAL TOXICITY

Rat **
** 299-0025; 273358; “Developmental Toxicity of Technical Bidrin Insecticide in Sprague-Dawley Rats”; (D.E. Rodwell; WIL Research Laboratories, Inc., Ashland, OH; Study No. WIL-93006; 6/25/86); Twenty five mated female Sprague-Dawley rats/sex/group were dosed orally by gavage with 0, 0.1, 0.5, 1.0 or 2.0 mg/kg/day of Bidrin technical (dicrotophos); no batch no.; purity: 89.7%) from day 6 through day 15 of gestation. The mean body weight gains of the dams in the 1.0 and 2.0 mg/kg treatment groups were less than that of the control group over the course of the treatment period. The 2.0 mg/kg group exhibited treatment-related clinical signs of teeth gritting, fasciculations, tremors, decreased muscle tone, nasal discharge, signs of diarrhea, urogenital staining and salivation. The 1.0 mg/kg dams also demonstrated the fasciculations. There were no apparent treatment-related effects upon the development of the fetuses. No adverse effect indicated. Maternal NOEL: 0.5 mg/kg/day (based upon the clinical signs, lower body weight gain and reduced food consumption noted for the 1.0 mg/kg treatment group); Developmental NOEL: 2.0 mg/kg/day (based upon the lack of a treatment-related effect upon the fetuses in the 2.0 mg/kg group); Study acceptable. (Moore, 10/8/13)

299-019 047154 This is a duplicate copy of study 299-0025; 273358, above.

Rabbit **
** 299-0026, -0027; 273359, 273360; “Dicrotophos: Prenatal Developmental Toxicity Study in the Rabbit:”; (M.E. Moxon; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. RB0865; 4/9/01); Twenty eight mated New Zealand White female rabbits/group were dosed orally by gavage with 0 (vehicle: water), 0.5, 1.0 or 2.0 mg/kg/day of Dicrotophos technical (batch no. 403001 B; purity: 87.65%) from gestation day 5 through gestation day 29. Two does in the 2.0 mg/kg group were euthanized in extremis on day 29 due to the severity of their clinical signs. One doe in the 1.0 mg/kg group was euthanized on day 30 following signs of an abortion. Clinical signs for the does in the 2.0 mg/kg treatment group included shaking, hunched posture, subdued behavior, increased breathing rate, abnormal respiratory noise, salivation, mucus in the feces, signs of diarrhea and staining in the genital area. For the does in the 1.0 mg/kg group, mucus was noted in the feces and there were signs of diarrhea. No treatment-related clinical signs were noted for the does in the 0.5 mg/kg group. The mean body weights of the 2.0 mg/kg does were less than the control group values at the initiation of dosing and during the last few days of gestation (p<0.05). The mean food consumption of the 1.0 and 2.0 mg/kg treatment groups was less than that of the control group during the last four days of the gestation period (p<0.01). The mean weight of the 2.0 mg/kg group fetuses was less than those in the control group (p<0.01). No adverse effect was evident. Maternal NOEL: 0.5 mg/kg/day (based upon the treatment-related clinical signs noted for the 1.0 mg/kg does); Developmental NOEL: 1.0 mg/kg/day (based upon the lower mean body weights noted for the fetuses in the 2.0 mg/kg group); Study acceptable. (Moore, 10/1/13)
299-017 036513 "Toxicity studies with Bidrin: Teratological studies in rabbits given Bidrin orally,” Tunstall Laboratory, Sittingbourne (presumably Kent, UK). Dix, K. M., A. B. Wilson, and W. V. McCarthy, Study TLGR.0020.73, Aug. 1973. Initially 32 control banded Dutch rabbits, or groups of 16 does administered 1.3 or 4.0 mg/kg/day Dicrotophos on gestation days 6-18, or positive control (16 dams administered 37.5 mg/kg/day thalidomide) were evaluated for developmental toxicity. These dose levels did not cause clear clinical signs. Three of 13 litters in the initial study administered 4 mg/kg/day dicrotophos had visceral abnormalities, prompting a repeat study. In the second study phase, 36 control does were compared to dicrotophos levels of 18 dosed with 1.3, 4, or (initially) 12 mg/kg/day. The latter dose proved too toxic: 3 of ten 12 mg/kg/day does died. Reduction of the highest dose in the second study phase to 8 mg/kg/day still found several clinical signs in the does, and one additional death. In the second phase, 2/21 control litters had visceral abnormalities, compared to none in dicrotophos groups (1.3, 4, or 8 mg/kg/day, with 12, 13, and 8 litters examined, respectively). Investigators justifiably concluded that dicrotophos was not a developmental toxicant under study conditions. Study pre-dated current guidelines, and lacked features such as QA oversight or dosing solution analysis, so that there is no DPR worksheet. Useful supplementary data. Aldous, Dec. 1, 2014.

NEUROTOXICITY

Acute neurotoxicity, rat ** † (flagged for brain AChE)

** 299-0032; 273379; “Dicrotophos: Acute Neurotoxicity Study in Rats”; (N.J. Rattray; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK1 0 4TJ; Study No. AR5795; 2/20/95); Ten Wistar rats/sex/group were dosed orally by gavage with 0 (distilled water), 0.5, 5 or 10 mg/kg of Dicrotophos technical (batch no. 403001B; purity: 87.65%). A satellite cohort of 10 animals/sex/group were dosed in the same manner. Five animals/sex/group/time point were euthanized at 3 hours or 8 days post-dose. Brain, red blood cell and plasma cholinesterase (ChE) activities were assayed. One male and six females in the 10 mg/kg group died within 3 hours of dosing. Clinical signs included decreased activity, ataxia, chromodacryorrhea, flaccidity, reduced foot withdrawal reflex, decreased pupillary response to light, salivation, shaking, sides pinched in, stains around mouth and nose, signs of urinary incontinence, tip toe gait and upward curvature of the spine. Most of these signs were demonstrated by both sexes in the 5 and 10 mg/kg treatment groups and were only evident on the day of dosing. The mean body weights of the 10 mg/kg males were less than those of the control group over the two-week observation period (p<0.01). The food consumption of these animals was also less than that of the control group during the first week post-dose. In the time to tail flick test, both sexes in the 5 and 10 mg/kg groups demonstrated a prolonged response time interval for the test on the day of dosing. In the grip strength assessment, the fore- and/or hindlimb grip strengths of both sexes in the 5 and 10 mg/kg groups were lower than those of the control group on the day of dosing (NS, p<0.05 or 0.01). Likewise, the motor activity of both sexes in the 5 and 10 mg/kg group was less than that of the control group animals on the day of dosing. None of these effects were evident in later functional observational battery or motor activity assessments. There was a significant reduction in brain cholinesterase activity for the animals in the 0.5 mg/kg and above on the day of dosing (p<0.01). The effect persisted in the 10 mg/kg males through the 1st week post-dose. Red blood cell and plasma cholinesterase activity levels for all of the treatment groups were also significantly reduced in comparison to the control
levels on the day of dosing. An effect was still evident on the red blood cell ChE activity of both sexes in the 10 mg/kg group at 1 week post-dose. The significant reduction of brain ChE activity at treatment levels for which plasma and red blood cell ChE activity levels are only marginally affected presents a major concern in regard to monitoring the activity levels of these two enzymes in worker safety programs. There was no apparent treatment-related effect noted in the necropsy or histopathological examinations. Possible adverse effect: reduced cholinesterase activity in the brain. ACUTE NEUROTOXICITY NOEL: (M/F) < 0.5 mg/kg (based upon the reduced brain cholinesterase activity noted for both sexes in the 0.5 mg/kg treatment group); Study acceptable. (Moore, 10/23/13)

90-day neurotoxicity, rat ** † (flagged for AChE inhibition)

** 299-0041; 276564; “Dicrotophos: Subchronic Neurotoxicity Study in Rats”; (S.A. Horner; Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Report No. CTL/P/4692; 11/6/95); Twelve Alpk:AP,SD rats/sex/group received 0, 0.5, 5 or 25 ppm of Dicrotophos technical (batch no. 403001B; purity: 87.65%) in the diet for 13 weeks ((M) 0, 0.04, 0.39, 2.03 mg/kg/day, (F) 0, 0.04, 0.45, 2.38 mg/kg/day). Two satellite cohorts of 6 animals/sex/group/cohort were treated in the same manner for 5 and 9 weeks, respectively. At those times, the animals were euthanized and plasma, red blood cell and brain cholinesterase (ChE) activities were assayed. The mean body weights and food consumption of both sexes in the 25 ppm group were less than the control values during the 1st weeks of the study (p<0.05 or 0.01). In the FOB, a decreased pupillary response was noted for 5 of 17 males and 2 of 18 females in the 25 ppm group at week 9. This was the only time point for which this effect was remarkable. The forelimb and hindlimb grip strength of the 25 ppm females was minimally reduced at week 9 (p<0.01 or 0.5). This effect was less apparent by week 14. Motor activity of both sexes in the 25 ppm group was reduced at week 9 and persisted through week 14 (NS, p<0.01 or 0.05). The ChE activity in the brain was reduced in both sexes of the 0.5 ppm treatment group and above (p<0.01 or 0.05). The plasma and red blood cell ChE activities were likewise reduced for both sexes in the 0.5 ppm treatment group at various time points during the study (p<0.01 or 0.05). There were no treatment-related lesions noted in the necropsy or histopathological evaluations. Possible adverse effect: significant reduction in brain ChE activity. Rat Subchronic Neurotoxicity NOEL: (M/F) < 0.5 ppm (0.04 mg/kg/day) (based upon the reduced cholinesterase activity in the brain of both sexes in the 0.5 ppm treatment group). Study acceptable. (Moore, 8/26/14) Another copy of this report was submitted in a subsequent submission package and under a different record number (Document No. 299-0064, Record No. 280958). The latter copy was evaluated by Aldous on 11/20/14. Conclusions by the two DPR reviewers were comparable, so only the above 1-liner is needed in this Summary.

Developmental neurotoxicity, rat **

** 299-0031; 273377; “Dicrotophos: Developmental Neurotoxicity Study in Rats”; (A. Brammer; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK1 0 4TJ; Study No. RR0884; 10/24/03); Thirty time-mated female Wistar rats/group were dosed orally by gavage with 0 (vehicle: deionized water), 0.01, 0.05 or 0.4 mg/kg/day of Dicrotophos technical (batch no. 403001B; purity: 87.65%) from day 7 gestation through day 7 post-partum. The pups in the F1 generation were dosed orally by gavage from day 8 through day 22 post-partum. A functional observational battery (FOB) was performed on the F0 dams on days 10 and 17 of gestation and on days 2 and 9 of lactation. For the F1 generation, the FOB was
performed on 10 pups/sex/group (one male or female from each litter) on post-partum days 5, 12, 22, 36, 46 and 61 in the same manner as it was performed with the dams. Motor activity was measured for one male and one female selected from each litter on days 14, 18, 22 and 60. The auditory startle response was assessed for one male and one female per litter on days 23 and 61 and the learning and memory was assessed using one male and one female from each litter on days 21 and 24 and 59 and 62. Two F0 females in the control group were euthanized due to parturition difficulties. There was no treatment-related effect upon the mean body weights of the F0 generation dams. The reproductive performance of the dams was not affected by the treatment. For the dams, no treatment-related clinical signs were evident in the FOBs performed over the course of the study. The treatment did not affect the mean body weights of the F1 generation offspring. The time to preputial separation or vaginal opening was not affected by the treatment. The F1 animals did not exhibit any treatment-related clinical signs in the FOBs or motor activity measurements performed. The startle response test did not demonstrate any apparent developmental deficits. There was no treatment-related effect in the learning and memory tests. In the necropsy examination, although the absolute brain weights of the pups in the 0.4 mg/kg group were statistically greater than those of the control group at either 12 or 63 days post-partum, there was no effect on the relative brain weights. No treatment-related lesions were noted in the histopathological examination. In the brain morphometric analysis, although certain of the measurements for the F1 offspring in the 0.4 mg/kg group were significantly different from that of the control group, no consistent effect on the brain structure was evident.

No adverse effect indicated. Maternal NOEL: 0.4 mg/kg/day) (based upon the lack of treatment-related effects on the dams in the 0.5 mg/kg treatment group); Developmental NOEL: 0.4 mg/kg/day (based upon the lack of a treatment-related effect on the development of the pups in the 0.4 mg/kg treatment group); Developmental Neurotoxicity NOEL: 0.4 mg/kg/day (based upon the lack of the treatment-related effect on the pups in the 0.4 mg/kg group); Study acceptable. (Moore, 10/18/13)

In a preliminary developmental neurotoxicity study (study no. RR883), reported in vol. no. 299-0031 under record no. 273377, dams experienced significant reduction in cholinesterase (ChE) activity in the RBC and brain of the dams at treatment levels of 0.05, 0.2 and 1.0 mg/kg/day. Fetal RBC and brain ChE activities were reduced on gestation day 22 at the 0.2 and 1.0 mg/kg/day treatment levels. The offspring did not demonstrate any ChE inhibition on lactation days 8, 15 or 22. In a second preliminary study (study no. KR1491), pre-weanling rats 12 days old or young adults 42 days old were dosed orally by gavage for 7 days with 0.008, 0.02, 0.08 or 0.4 mg/kg/day. Reduced brain and RBC ChE activities were noted for the pre-weanlings and young adults treated with 0.4 mg/kg/day. The pre-weanlings also demonstrated reduced RBC ChE activity at 0.08 mg/kg/day. Based on these results, 0.01, 0.05 and 0.4 mg/kg/day were selected as the treatment levels for the guideline study.

299-0031; 273378 This is an analysis of brain morphometry, sent as a response to a U.S. EPA request. It is a few pages in length, and should be considered part of Record No. 273377.

Delayed neurotoxicity, hen **

** 299-0033; 273380; “Dicrotophos: A Delayed Neurotoxicity Study in Laying Hens Phase II-Acute Neurotoxicity Assessment”; (L.T. Frey, J.B. Beavers, K.H. Martin, M.J. Jaber; Wildlife International, Ltd., Easton, MD; Project No. 246-112; 7/7/00); Twenty Single comb, white
Leghorn hens were dosed orally by intubation with 11 mg/kg of Dicrotophos technical (lot no. 8070030051; E isomer: 87.2%, Z isomer: 6.2%). Twelve hens/group were dosed in the same manner with either 0 (reverse osmosis water) or 600 mg/kg of tri-orthocresyl phosphate (TOCP) in corn oil. The hens treated with dicrotophos were also given intramuscular injections of atropine (0.5 mg/kg) and 2-PAM (50 mg/kg) immediately prior to dosing, once later in the day, 3 times on day 1 and three additional injections of atropine on day 2. The hens in the dicrotophos treatment group demonstrated acute symptoms of toxicity; lethargy, loss of coordination, wing droop, reduced reaction to external stimuli, lower limb weakness and depression. These signs were first noted on day 1 and continued in at least one bird until day 8. Thereafter, no signs were evident. These hens demonstrated a loss in body weight during the first week post-dose, thereafter regaining the weight. The food consumption of these birds was reduced for the first week in comparison to the control group, recovering to the control level for the remainder of the study. In the ataxia assessment, the dicrotophos-treated birds demonstrated some acutely toxic effects during the first week, largely recovering during the second week. The positive control cohort, the TOCP-treated birds, did not demonstrate the delayed neurotoxic deficit as expected. Neurotoxic esterase (NTE) activity in the brain and spinal cord of the dicrotophos-treated hens was 92 and 75% of the control values, respectively, at 2 days post-dose. The TOCP- treated hens demonstrated activity levels of 9 and 13% of the control values for the brain and spinal cord, respectively. The brain acetylcholinesterase activity in the dicrotophos-treated hens was only 16% that of the control group in contrast to that of the TOCP-treated birds which was 79% of control. The histopathological evaluation did not reveal any treatment-related lesions in either the dicrotophos- or TOCP-treated hens. These results confirmed the lack of treatment-related effects in the ataxia assessment. No adverse effect indicated. The positive control was not fully functional, no delayed neuropathy was manifested. Despite this result, there was sufficient information to substantiate that dicrotophos is not a delayed neurotoxicant. Study acceptable. (Moore, 10/24/13).
IMMUNOTOXICITY **

** 299-0035; 273382; “Dicrotophos Technical: 4 Week Dietary Immunotoxicity Study in the Male Han Wistar Rat”; (W. Arrowsmith; Huntingdon Life Sciences Ltd., Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England; Project ID No. BDG0003; 2/3/11); Ten male Wistar rats/group received 0, 5, 15 or 25 ppm of Dicrotophos technical; batch no. GB101309-01; purity: 88.9% (E-isomer: 85.4%, Z-isomer: 3.5%) in the diet for 4 weeks (0, 0.37, 1.14, 1.91 mg/kg/day). Another 8 males were dosed by intraperitoneal injection with 50 mg/kg of cyclophosphamide in 0.9% saline on day 27 as the positive control group. On day 25, five days before necropsy on day 29, each animal received an iv injection of 2x10^8 sheep red blood cells (SRBC). SRBC-specific IgM plaques were determined for each animal by incubating a spleen cell suspension preparation with guinea pig complement and SRBC. No deaths occurred during the treatment period. The mean body weight gain of the 25 ppm animals was less than that of the control group over the course of the study (p<0.01). Brain and red blood cell cholinesterase activities were reduced in a dose-related manner in all of the treated groups (p<0.01). In the necropsy examination, the adjusted spleen weight of the 25 ppm males was greater than that of the control group (p<0.05). This greater weight was reflected in the greater numbers of cells/spleen and plaque-forming cells/spleen determined in the plaque forming assay. There was no treatment-related effect evident in the plaque-forming cell assay. No adverse effect indicated. The positive control was functional. Study acceptable. (Moore, 10/25/13)

ENDOCRINE DISRUPTOR STUDIES

No study submitted nor required at this time.

MECHANISTIC STUDIES (largely acetylcholinesterase inhibition)

299-0067 280961 Moxon, M. E., “Dicrotophos: acute cholinesterase inhibition study in pre-weaning rats,” Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, 10/24/03. Laboratory Study # CTL/AR7148/Regulatory/Report. Groups of 5 pups/sex were dosed by gavage once with Dicrotophos Technical, 90.4% purity, Batch 403001B at three ages (PND 8, 15, and 22), and at 5 dose levels (0, 0.1, 0.3, 1, and 5 mg/kg). Pups were killed about 2 hrs after dosing for assays of brain and RBC AChE. All 5 mg/kg pups suffered tremors. Additional characteristic signs of AChE inhibition were seen in 5 mg/kg pups: most evident at PND 15. Clinical signs at lower dose levels were limited to one 1 mg/kg pup with slight tremors. Well-defined and statistically significant brain AChE inhibition dose-responses were observed for both sexes and all ages of pups over the dose range from 0.3 to 5 mg/kg dicrotophos. Also, brain AChE in 0.1 mg/kg pups was slightly below controls, generally also significantly significant. Regardless of sex, well-defined RBC AChE inhibition dose-responses were observed for all ages of pups over the dose range from 0.3 to 5 mg/kg Dicrotophos (statistically significant except for PND 8 females). At PND 15 and PND 22, RBC AChE in 0.1 mg/kg pups was appreciably below controls, generally also significantly significant. In contrast, there was no decline in RBC AChE in PND 8 pups at 0.1 mg/kg. This supplementary study did not seek and did not find a NOEL, however useful dose-response patterns were revealed, so that the study provides valid supplementary data. Aldous, 11/17/14.
299-0068 280962 Brammer, A., “Dicrotophos: acute cholinesterase inhibition study in rats,” Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, 4/4/02. Laboratory Study # AR7078. Groups of 5 Alpk:AP:SD rats/sex/[sacrifice group] were dosed once by gavage with Dicrotophos [87.6% purity, Batch 403001B] at dose levels of 0, 0.1, 0.3, and 5 mg/kg and sacrifice times of 3 hours on day 1, and on days 8 and 15. Prominent clinical signs, all limited to 5 mg/kg rats, included tremors, decreased activity, splayed gait, reduced stability, sides pinched in, spine curved upward, and irregular breathing. All of these signs were limited to the first treatment day. Slight decreases in day 8 body weights for 5 mg/kg males and small food consumption reductions in 5 mg/kg females during week 1 may also have been treatment-related. Day 1 brain AChE activity was reduced in 5 mg/kg males by 74%, with no measurable effect at 0.3 mg/kg. Day 1 brain AChE activity was reduced in 5 mg/kg females by 76%, and there was a 22% reduction at 0.3 mg/kg. There was an equivocal brain AChE activity reduction in 5 mg/kg females at day 8 (18% below concurrent control). Day 1 RBC AChE activities were reduced in dose-related fashion, statistically significantly so in males and females at 0.3 and 5 mg/kg. Percent reductions were 15% and 47%, respectively, in males; and 10% and 39%, respectively, in females. There were no RBC AChE changes at later sacrifice times. NOEL for parameters assessed in this study was thus 0.1 mg/kg. Useful supplementary data. Aldous, 11/18/14.

299-0065 280959 Brammer, A., “Dicrotophos: repeat dose bridging study in rats,” Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, 2/18/02. CTL Laboratory Study No. KR1455. Groups of 5 rats/sex/group were dosed by gavage daily for 28 days or 56 days in a study to assess clinical signs and cholinesterase (ChE: brain and RBC) effects of Dicrotophos, 87.6% purity, Batch 403001B. Dose levels were 0 or 0.4 mg/kg/day. Investigators focused on possible accumulated effects, rather than on peak effect after bolus dosing. Rats were examined pre-test and just before daily dosing for clinical signs. Necropsy (mainly for brain and RBC sampling) was one day after final dosing respective groups. No clinical signs were evident when examined (nearly 24 hours since the previous day’s dose). Body weights were marginally decreased by study termination in both sexes. Brain and RBC ChE activities did not vary by sex, and inhibition did not change significantly between the 4-week and the 8-week treatment regimen. Study provides useful supplementary data, with some deficiencies in the report. Aldous, 11/20/14.

299-0039; 276562; “Dicrotophos: Repeat Dose Cholinesterase Inhibition Study in Rats”; (A. Brammer; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. KR1456; 6/24/02); Ten Wistar-derived rats/sex/group were dosed orally by gavage with 0 (vehicle: deionized water), 0.008, 0.02 or 0.4 mg/kg/day of Dicrotophos technical (batch no. 403001B; purity: 87.6%) for 28 days. Five animals/sex/group in the main study were euthanized at the conclusion of dosing and brain and red blood cell acetylcholinesterase (AChE) activities were measured. A recovery cohort of 5 animals/sex/group were maintained treatment-free for an additional 4 weeks. At that time the animals were euthanized and the brain and RBC AChE activities were assayed. No test material-related deaths occurred during the study. There were no apparent treatment-related clinical signs or effects on mean body weight. In the main study group, the brain AChE activity levels of both sexes in the 0.4 mg/kg treatment group and the females in the 0.02 mg/kg group were reduced in comparison to the control group values (NS or p<0.01). In the recovery cohort, the female brain AChE activity levels were still reduced for all of the treated groups after 4 weeks. This persistence may have been due to an exceptionally high
control activity level. However, this potential effect bears further evaluation. **Possible adverse effect**: significant reduction in brain AChE activity; **Rat 4-Week Oral Toxicity NOEL**: (M) 0.02 mg/kg/day (based upon reduced brain AChE activity in the 0.4 mg/kg treatment group; (F) 0.008 mg/kg/day (based upon the reduced brain AChE activity in the 0.02 mg/kg treatment group); **Study supplemental**. (Moore, 8/8/14). Another copy of this report was submitted in a subsequent submission package and under a different record number (Document No. 299-0066, Record No. 280960). The latter copy was evaluated by Aldous on 11/20/14. Conclusions by the two DPR reviewers were comparable, so only the above 1-liner is needed in this Summary.

299-0069 280963 Moxon, M. E., “Dicrotophos: repeat dose cholinesterase inhibition study in pre-weaning and young adult rats,” Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, 10/24/03. CTL Study # KR1491. Alpk:APfSD rats, 5/sex/group, were dosed by gavage for 7 consecutive days with Dicrotophos [90.4% purity, Batch 403001B] at 0, 0.008, 0.02, 0.08, 0.4, or 1 mg/kg/day. This regimen applies to both pre-weaning and young adults. For pre-weanlings, dosing was PND 12-18. For young adults, dosing was PND 42-48. The primary assessments were of brain and RBC acetylcholinesterase (AChE): in all cases assessed after sacrifice 2 hrs following the last treatment. NOEL = 0.02 mg/kg/day for RBC AChE in pre-weanling rats. The NOEL for brain and RBC AChE in young adult rats is 0.08 mg/kg/day, as is the NOEL for brain AChE in pre-weanling rats. In all cases, inhibition was strong at 0.4 mg/kg/day and above in pre-weaning and in young adult rats, with inhibition typically slightly greater in pre-weaning rats. There were no clinical signs at any dose tested. Useful supplementary data. Aldous, 11/21/14.

299-0062; 280094; “Dicrotophos: 14 Day Dermal Toxicity Study in the Rat with Cholinesterase Determination”; (I.R. Johnson; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK SK10 4TJ; Study No. LR0589; 12/21/00); The skin of 5 Crl:CD rats/sex/group was exposed to 0 (deionized water), 2, 5, or 10 mg/kg/day of Dicrotophos technical (batch no. 403001B; purity: 87.6%) for 6 hours/day for 14 days. Upon the completion of this treatment regimen, the animals were maintained for another 2 weeks without treatment. Cholinesterase activity was assayed in the red blood cells and plasma of these animals on study days 2, 8, 15, 22 and 29. No deaths resulted from the treatment. No treatment-related clinical signs were evident. The mean body weights were not affected by the treatment. Red blood cell cholinesterase activity was 83 and 77% of the control group for the males in the 5 and 10 mg/kg treatment groups, respectively after 8 days of treatment (p<0.01). For the females, the maximal reduction in red blood cholinesterase was noted after 14 days of treatment for the 10 mg/kg treatment group (71% of control, p<0.01). For plasma cholinesterase activity, a maximal reduction for the males was evident after 14 days of treatment in the 5 and 10 mg/kg treatment groups (71 and 72% of control, respectively, p<0.01). A reduction in activity was still evident up to 7 days post-final treatment. For the females, maximal reduction in plasma cholinesterase activity was evident by study day 8 for the 5 and 10 mg/kg groups (64 and 55% of control, respectively, p<0.01). The effect persisted through study day 15. **No adverse effect indicated. NOEL** was not established due to the limitation of the evaluated data. **Study supplemental** (non-guideline study). (Moore, 9/22/14)

which pre-dated current guidelines, and which had no QA oversight, Bidrin was administered to leghorn hens at 8 mg/kg to assess possible demyelination. The hens had been pre-treated with atropine and protopam chloride (pralidoxime chloride) to protect against acute toxicity. Eight of the 12 dosed hens survived, and were sacrificed after 3 weeks. Unspecified nerves were examined histologically, and no demyelination was evident. Since this report did not indicate adverse effects and could not be upgraded, no DPR worksheet is needed. Aldous, 12/1/14.

299-017 036520 Witherup, S., K. L. Stemmer, and H. Schlecht, “Specific physiological effects of Bidrin ®, Vapona ®, and Ciodrin ® insecticides in chickens,” The Kettering Laboratory, Cincinnati, OH, Nov. 25, 1963. This is a brief report of a study evaluating possible delayed neuropathy due to 3 organophosphorus insecticides. The study design was free-form, and pre-dated current guidelines, hence is supplementary data. After a series of treatments to determine survivable dose levels, the definitive Bidrin delayed neuropathy study was conducted with 14 hens, each treated twice on day 1 with 1.5 mg/kg/dose, followed by a 1-week resting phase. Then in weeks 2 and 3, each hen received 0.75 mg/kg/day for 5 days each week. Clinical signs after the week 1 high dose exposures included weakness, unsteadiness, tremors, muscle fasciculations, diarrhea, salivation, lacrimation, and sometimes labored respiration and collapse. Following the lesser dosing during weeks 2-3, signs were limited to weakness and unsteadiness in several hens, with occasional observations of tremors and/or muscle fasciculations. At necropsy, at least some peripheral nervous and brain sections were examined for potential neuropathies. No neurohistopathology was associated with Bidrin or other insecticides evaluated, whereas positive controls (TOCP and trimethylphosphate) elicited varying degrees of peripheral demyelination and neurophagia in the brain cortex. Useful supplementary information. No DPR review is relevant. Aldous, Dec. 2, 2014.