

APPENDIX 1.

SUMMARY OF TOXICOLOGY FOR CHLORPYRIFOS

Updated April 20, 2018

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
HUMAN HEALTH ASSESSMENT BRANCH

SUMMARY OF TOXICOLOGY DATA
CHLORPYRIFOS

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DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, no adverse effect
Developmental toxicity, rat:	No data gap, no adverse effect
Developmental toxicity, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, possible adverse effect
Neurotoxicity:	No data gap, no adverse effect

Toxicology one-liners are attached.

All record numbers for the above study types through 299293 (Document No. 342-1014) were examined. This includes all relevant studies indexed by DPR as of April 10, 2018.

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.

File name: t20180420 chlorpyrifos

Current revision by C. Aldous, April 20, 2018

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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HAZARD IDENTIFICATION SUMMARY FOR CHLORPYRIFOS

Metabolism: Chlorpyrifos was efficiently absorbed by rats following gavage dosing of chlorpyrifos in corn oil, as indicated by approximately 90% of a labeled dose being found in urine. Humans absorbed about 72% of an oral dose from a lactose tablet, compared to about 1.35% of a dermal dose. About 50% of administered dose was captured in urine of rats within 12 hours of dosing. Major urinary metabolites in rats and in humans were 3,5,6-trichloro-2-pyridinol (TCP) and (at least in rats) its glucuronide conjugation products. The elimination half-life of TCP in humans is about 27 hours, making TCP concentration a rough indicator of recent chlorpyrifos exposure. Oral absorption in humans dosed with 0.5 to 2 mg/kg chlorpyrifos in gelatin capsules was 30-35%. Generally, the low doses used in human and monkey studies found blood chlorpyrifos levels near to the limits of detection. A rat study with single oral dose levels of 0.5, 1, 5, 10, 50, and 100 mg/kg chlorpyrifos, found peak (3-hour) blood levels of chlorpyrifos of 3, 30, 113, 444, and 798 ng/g blood at 1 to 100 mg/kg, respectively (not detectable at 0.5 mg/kg). Estimated half-life for chlorpyrifos in blood was 2.7, 1.5, 2.1, or 7.3 hours for 5, 10, 50, or 100 mg/kg chlorpyrifos dose levels, respectively. In the same study, chlorpyrifos oxon was detected at a maximum of 2.5 ng/g blood, this being 1 hour after dosing with 50 mg/kg chlorpyrifos.

Acute Toxicity: Oral dosing found rat LD₅₀ of 144-223 mg/kg, with clinical signs at high doses such as fecal soiling, lacrimation, urine soiling, salivation, and decreased activity. Dermal LD₅₀ was greater than 5000 mg/kg, with limited clinical signs (soiled fur). Inhalation LC₅₀ was over 4.07 mg/L (male) and 2.87 mg/L (female), accompanied by clinical signs similar to those of oral dosing. Primary eye irritation and primary dermal irritation studies showed mild effects (Category III and IV). Chlorpyrifos is not a sensitizer.

Subchronic Toxicity: Available subchronic studies were generally performed as pilot studies for longer-term studies, or to evaluate cholinesterase (ChE) effects (reported separately in this section). The subchronic rat study found slight ChE reduction (in plasma ChE) at 0.1 mg/kg/day, even though only limited ChE-related clinical signs could be found at a much higher dose (10 mg/kg/day). The dog subchronic study found that about 50% brain ChE inhibition was observed at 200 ppm, and gross cholinergic symptoms were observed at 600 ppm.

Chronic Toxicity and Oncogenicity: A lifetime rat oncogenicity study (Record No. 153114) reported findings at 100 ppm including modest body weight decrements and over 50% brain ChE inhibition in both sexes, and an increase over baseline incidences of diffuse retinal atrophy and cataracts in 100 ppm females. Associated overall achieved dose levels were in the range of 5 to 6 mg/kg/day for males and 6 to 7 mg/kg/day for females. The latter dose did not elicit definitive cholinergic signs such as were reported in acute oral testing, above. A mouse oncogenicity (79-week) study found severe brain ChE inhibition at 250 ppm (residual brain ChE activity about 20% or less in both sexes), without clearly-associated cholinergic signs. That study achieved dose levels of 45-46 mg/kg/day in either sex at 250 ppm midway through the study. There were no treatment-related tumors in either species.

Genotoxicity: mutation studies in bacteria and mammalian cells were negative, as were cytogenetics assays. An acceptable unscheduled DNA synthesis (UDS) assay was negative. Two studies designed to evaluate DNA damage were reportedly positive, but could not be fully evaluated by DPR because the underlying data were not available. The positive findings of the DNA damage tests thus cannot be dismissed at this time.

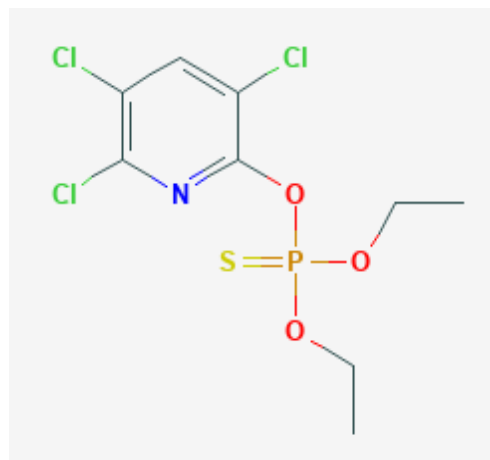
Reproductive and Developmental Studies: The reproduction study found a statistically significant reduction in pup weights in the first generation, and a slight reduction in pup survival in the second generation, both at 5 mg/kg/day. Pup losses tended to be specific to particular litters, often associated with signs of maternal neglect, such as multiple pups which were weak, pale, cold, or with no milk in the stomach. As maternal brain ChE at 5 mg/kg/day was severely inhibited (51% of control in F0 dams and 42% of control in F1 dams), the findings in pups were attributed to maternal toxicity. Two valid rat developmental toxicity studies dosed the dams up to a maternally toxic level (tremors at 15 mg/kg/day). One study was negative for developmental effects, and the other study reported a slight increase in early resorptions at that dose. Neither of these studies was considered “adverse” with respect to developmental toxicity. A rabbit developmental toxicity study found maternal body weight gain decrements at 140 mg/kg/day, associated with developmental delays in fetuses. There were no effects on either dams or fetuses at the next lower dose of 81 mg/kg/day. No adverse effects were indicated. An acceptable mouse developmental toxicity study found slight developmental delays at 25 mg/kg/day, with a NOEL of 10 mg/kg/day. This was not considered to be “adverse,” considering that the dams had clinical signs of tremors and excessive salivation at 10 and 25 mg/kg/day.

Neurotoxicity: An acute neurotoxicity study found transitory effects shortly after dosing: reduced body weights and perineal soiling at 50 and 100 mg/kg/day, in addition to FOB observations of incoordination, decreased muscle tone, tremor, increased lacrimation and salivation at 100 mg/kg/day in females immediately after dosing on day 1. Motor activity was reduced at 50 and 100 mg/kg/day on day 1; some reductions persisted to day 8 in 100 mg/kg/day females. NOEL

was 10 mg/kg. There were no histopathologic changes. Findings were not considered to be “adverse” in the context of the study objectives. A 90-day neurotoxicity study found reduced motor activity at 15 mg/kg/day at observation week 4, but not subsequently. Perineal soiling was occasionally observed at 5 and 15 mg/kg/day. There were no neurohistopathological findings. In the absence of substantial or progressive changes, this study was not considered to indicate “adverse” effects. A developmental neurotoxicity study dosed dams from gestation day 6 through lactation day 11. Maternal brain ChE activity at gestation day 20 was inhibited by 90% at 5 mg/kg/day, and by 18% at 1 mg/kg/day. Dams displayed clinical signs during gestation (fasciculations), and additionally hyperreactivity and hyperpnea at lactation at 5 mg/kg/day, but not at lower dose levels. Pups suffered early neonatal losses, body weight losses, and developmental delays at 5 mg/kg/day, with no changes at 1 mg/kg/day. Considering the extreme toxicity to the dams at 5 mg/kg/day, no findings in offspring were of sufficient magnitude to designate the study as “adverse” with respect to offspring.

Immunotoxicity: A valid immunotoxicity study found no adverse effects.

Cholinesterase (ChE) Inhibition: Plasma cholinesterase (ChE) is a relatively sensitive indicator of recent chlorpyrifos exposure (i.e., a few hours). Male human volunteers administered a 0.5 mg/kg single oral dose of chlorpyrifos had plasma ChE inhibited to about 15% of baseline, with maximal inhibition at 0.5 to 2 hrs after dosing. By 8 hours, plasma ChE levels had substantially recovered. By 27 to 30 hours, plasma ChE activity had returned to baseline. RBC ChE was not measurably inhibited at 0.5 mg/kg, but appeared to have been inhibited in a human subject following a single oral dose of 2 mg/kg in another study. In a gavage single dose study in rats, brain ChE inhibition was evident at 10 mg/kg and above, with brain ChE activity (as percent of control) at 6-hour peak response being 88%, 30%, and 28% in 10, 50, and 100 mg/kg groups, respectively.



Chlorpyrifos

Active Ingredient structure from <https://pubchem.ncbi.nlm.nih.gov>

METABOLISM AND PHARMACOKINETICS ** (based on collective data)

NOTE: A number of studies in the Miscellaneous section near the end of this Summary include metabolism, pharmacokinetics, and cholinesterase inhibition data.

342-0343 071390 Nolan, R. J., M. D. Dryzga, B. D. Landenberger, and P. E. Kastl, "Chlorpyrifos: tissue distribution and metabolism of orally administered ¹⁴C-labeled chlorpyrifos in Fischer 344 rats," The Dow Chemical Company, Midland, MI, 12/23/87. Laboratory Study # K-044793-(76). Five rats/sex/group were dosed by gavage in 2 ml/kg corn oil in single labeled doses of 0.5 or 25 mg/kg or 15 consecutive daily doses of unlabeled chlorpyrifos at 0.5 mg/kg/day, followed 1 day after the 15th dose with a single labeled dose of 0.5 mg/kg. Labeled chlorpyrifos (>99% radiopurity) was 12 µCi per gram of corn oil regardless of dose. Only the 3,5,6-trichloro-2-pyridinol group was labeled. Unlabeled chlorpyrifos, used to dilute the high dose group, was 99.9% purity. Investigators evaluated label in urine, feces, and tissues, and identified the three significant urinary metabolites. Urine plus cage wash accounted for 86 to 93% of administered label, regardless of sex or dosing regimen. Six to 11% of label was found in feces. Urinary excretion was rapid: usually over 50% of administered dose was collected in urine within the first 12 hours (T_{1/2} was 8-9 hours for single or multiple 0.5 mg/kg treatments, and somewhat longer for 25 mg/kg rats). Urinary metabolites were composed chiefly of 3,5,6-trichloro-2-pyridinol, and usually slightly more of its glucuronide, collectively accounting for over 90% of urinary metabolites. About 5% of urinary residues consisted of the sulfate conjugate of 3,5,6-trichloro-2-pyridinol. Parent chlorpyrifos was not found in urine. Most fecal label was obtained within the first 24 hours. Exhaled CO₂ was trapped for radioanalysis from the 25 mg/kg group. This collection accounted for <0.01% of administered dose. Fecal metabolites were not assessed. Tissue residues were assessed at 72 hrs (M) and at 144 hrs (F). Total tissue residues were very small (0.2% of administered dose in 25 mg/kg group) to negligible (<0.01%), and generally only quantifiable in peri-renal fat (M and F). In the 25 mg/kg groups only, tiny but quantifiable residues were also found in liver (M) and ovaries. This is a valid supplementary study. Aldous, June 5, 2015.

GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

Acute oral toxicity, rat **

**342-716; 154442; Stebbins, K. E., "Dursban F Insecticidal Chemical: Acute Oral Toxicity Study in Fischer 344 rats," study type 811; The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI; Study No. K-044793-102A; 11/27/96; Dursban F Insecticidal Chemical (purity: 97.6%); 5 animals/sex/group; Doses: 50, 100, 500 mg/kg as 3% suspension in 0.5% aqueous solution of Methocel A4M; Mortality: 50 (M/F:0/5), 100 (M/F:0/5), 500 (M/F:5/5), deaths occurring with 3 days after dosing; Clinical Observations: fecal soiling, lacrimation, urine soiling, salivation, decreased activity; Necropsy: no treatment-related lesions noted; LD50 (M/F): 223 mg/kg; Toxicity Category II; Study acceptable. (Moore, 5/29/97)

**342-708; 154314; Nissimov, S. and A. Nyska, "Pyrinex Tech.: Acute Oral Toxicity in the rat," study type 811; Life Science Research Israel Ltd., Ness Ziona 70451, Israel; Study No. MAK/056/PYR; 5/12/84; Pyrinex Tech; 5 animals/sex/group; Doses: 90, 164, 298, 543, 987 mg/kg, in corn oil; Mortality: 90 (M/F:0/5), 164 (M:0/5, F:4/5), 298 (M/F:5/5), 543 (M/F:5/5), 987 (M/F:5/5); Clinical Observations: tremors, hunched posture, salivation, diarrhea, decreased motor activity, ataxia; Necropsy: hemorrhagic and/or ulcerated stomach and intestines; LD50

(95% confidence interval): (M) 221 (181 to 269) mg/kg, (F) 144 (105 to 200) mg/kg; Toxicity Category II; Study acceptable. (Moore, 6/10/97)

Acute dermal toxicity **

**342-716; 154444; Stebbins, K. E., “Dursban F Insecticidal Chemical: Acute Dermal Toxicity Study in New Zealand White Rabbits,” study type 812; The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI; Study No. K-044793-102D; 11/27/96; Dursban F Insecticidal Chemical (purity: 97.6%); 5 animals/sex/group; Doses: 2000, 5000 mg/kg, test material liquefied prior to application, 24 hour exposure; No mortality; Clinical Observations: fecal soiling, dermal irritation at the site of application; Necropsy: no treatment-related lesions; LD50 (M/F) > 5000 mg/kg; Toxicity Category IV; Study acceptable. (Moore, 5/30/97)

**342-709; 154315; Nissimov, S. and A. Nyska, “Pyrinex Tech.: Acute Dermal Toxicity in rabbits,” study type 812; Life Science Research Israel Ltd., Ness Ziona 70451, Israel; Study No. MAK/059/PYR; 5/12/84; Pyrinex Tech; 5 animals/sex; Dose: 2000 mg/kg, liquefied prior to application, 24 hour exposure, semi-occlusive wrap; No mortality; Clinical Observations: no treatment-related signs; Necropsy: congested lungs, skin lesions, multiple petechiae on thymus; LD50 (M/F) > 2000 mg/kg; Toxicity Category III; Study acceptable. (Moore, 6/10/97)

Acute inhalation toxicity, rat **

**342-710; 154316; Buch, S. A., “Pyrinex Tech.: Acute Inhalation Toxicity in rats,” study type 813; Life Science Research, Stock, Essex, England; Study No. 80/MAK025/362; 8/27/80; Pyrinex Tech (purity: 95.%); 5 animals/sex/group unless otherwise noted; Exposure Concentrations (gravimetric): 1.69 (F only), 2.23, 2.98, 3.56, 4.07 mg/l, MMAD (GSD): 7.4 (2.2), 7.9 (1.7), 8.2 (1.9), 8.0 (2.0), 8.6 (2.1) μm , respectively, respirable concentration (mass of particles < 10 μm): 1.40, 1.86, 2.61, 3.01, 3.47 mg/l, respectively, 4 hour nose-only exposure (test material was prepared as a 60% (w/v) in xylene) (concentrations based upon non-volatile portion of exposure atmosphere); Mortality: 1.69 (F:1/5), 2.23 (M:0/5, F:2/5), 2.98 (M:0/5, F:3/5), 3.56 (M:0/5, F:2/5), 4.07 (M:0/10, F:4/5); Clinical Observations: decreased motor activity, hunched posture, ataxia, tremor, hypothermia, piloerection, pigmented stain around eye and snout, gasping, bradypnea, muscle fasciculations; Necropsy: lungs pale and/or congested, liver pale with accentuation of lobular pattern, increased relative lung weights among the decedents; LC50 (95% confidence limit): (M) > 4.07 mg/l, (F) 2.89 (2.01 to 4.16) mg/l; Toxicity Category III; Study acceptable. (Moore, 6/11/97)

342-343; 71387; Landry, T. D., D. A. Dittenber, L. G. Lomax, and J. J. Momany-Pfruender, “Chlorpyrifos: an acute vapor inhalation toxicity study with Fischer 344 rats,” study type 813; Dow Chemical Company, Mammalian and Environmental Toxicology Research Laboratory, Midland MI; Lab Study No. K-44793-74; 12/3/86; Chlorpyrifos (Reference No. AGR 219646; purity = 100%), used neat; 0 (air) (24M/24F), 3.5 (6M/6F), 6 (12M/12F), 14 (6M/6F) ppm (analytical); vapor inhalation, 6-hour, whole-body and nose-only exposures; Mortality- one male at 6 ppm (attributed to physical trauma); Clinical Observations- reduced plasma cholinesterase activity (13-24% reduction) in 6 ppm group only (attributed to oral ingestion or dermal absorption of the dose); hyperactivity (considered not exposure-related); Necropsy- no treatment-related findings; reported LC50 (M and F) > 14 ppm (0.22 mg/l); Supplemental. (Duncan, 6/21/91)

Primary eye irritation, rabbit **

**342-716; 154445; Stebbins, K. E., “Dursban F Insecticidal Chemical: Primary Eye Irritation Study in New Zealand White Rabbits,” study type 814, The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI; Study No. K-044793-102C; 11/27/96; Dursban F Insecticidal Chemical (purity:97.6%); 6 animals; Dose: 0.1 ml/eye, liquefied prior to application; Observations: no ocular irritation evident at 24 hours; Toxicity Category IV; Study acceptable. (Moore, 5/30/97)

**342-711; 154317; Buch, S. A. and J. R. Gardner, “Pyrinex Tech.: Irritance to rabbit eye,” study type 814; Life Science Research, Stock, Essex, England; Study No. 80/MAK023/143; 4/30/80; Pyrinex Tech; 6 animals (eyes not rinsed); Dose: 100 mg/eye; Observations: no corneal opacity nor iritis evident, Conjunctiva (redness)-grades 2 (1/6) and 1 (5/6) at 24 hours, grade 1 (1/6) through 7 days (termination), no chemosis nor discharge evident at 24 hours; Toxicity Category III; Study acceptable. (Moore, 6/11/97)

Primary dermal irritation **

**342-716; 154446; Stebbins, K. E., “Dursban F Insecticidal Chemical: Primary Dermal Irritation Study in New Zealand White Rabbits,” study type 815; The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI; Study No. K-044973-102B; 11/27/96; Dursban F Insecticidal Chemical (purity: 97.6%); 6 animals; Dose: 0.5 ml/site, liquefied prior to application, 4 hour exposure; Observations: erythema-grade 1 (6/6) at 30 minutes post-exposure, grade 1 (4/6) at 24 hours, grade 1 (2/6) at 48 and 72 hours, clear by 7 days; Toxicity Category IV; Study acceptable. (Moore, 5/30/97)

**342-712; 154319; Buch, S. A. and J. R. Gardner, “Pyrinex Tech.: Irritance to rabbit skin,” study type 815; Life Science Research, Stock, Essex, England; Study No. 80/MAK024/144; 4/30/80; Pyrinex Tech; 6 animals; Dose: 0.5 gm/site (4 sites, 2 intact, 2 abraded), moistened with 0.2 ml of physiological saline, 23 hour exposure, occlusive wrap; Observations: (intact sites) erythema-grades 2 (3/6) and 1 (3/6) at 24 hours post-dosing, grade 1 (1/6) at 72 hours and on day 8, edema-grade 1 (1/6) at 24 hours post-dosing, clear by 72 hours; Toxicity Category IV; Study acceptable. (Moore, 6/11/97)

Dermal sensitization **

**342-0716 154447 Stebbins, K. E., “Dursban F Insecticidal Chemical: Dermal Sensitization Potential in Hartley Albino Guinea Pigs,” The Dow Chemical Company, Midland, MI, 11/27/96. Laboratory Study # K-044793-102E. Investigators first determined that the lowest non-irritating dose of Dursban F was 1% in dipropylene glycol monomethyl ether (DPGME). This dose level was used in the primary study. In all sensitization cases, induction was performed weekly for 3 weeks, and challenge followed two weeks after the third induction (with skin site examination 24 and 48 hrs after challenge). On each occasion, 0.4 ml of material was applied to clipped, intact skin for 6 hours. Test materials for positive controls were either DER 331 epoxy resin (neat) or dinitrochlorobenzene (DNCB, 0.5% in DPGME vehicle). Groups of five naïve animals were dosed twice (one week apart) with each of the three treatments as non-induced controls. Under these circumstances, Dursban F induction/challenge group showed erythema in only one animal (the same animal showing “slight” erythema during induction week 1 and again “slight” erythema 48 hrs after challenge). Main study positive controls were uniformly negative for skin irritation during the first two induction treatments, then frequently showed “slight” erythema at

the third induction treatment. Both positive controls typically displayed “slight” to “moderate” erythema at challenge. Treatments of naïve animals were uniformly negative, except for one Dursban F animal with “slight” erythema. Thus test system was viable, and **negative** for dermal sensitization for Dursban F. Study is **acceptable**, with no adverse effects. Aldous, 4/14/15.

342-0713 154320 Berman, C. L., “Evaluation of Chlorpyrifos (Pyrinex) for dermal sensitization of guinea pig,” Arthur D. Little, Inc., Cambridge, MA, 10/21/1987. Test article was chlorpyrifos, 96.8% purity, Technical grade. This study was examined on 7/29/97 by C. Rech of DPR, who noted several deficiencies, and requested a replacement study. This unacceptable study did not indicate sensitization potential. (Aldous, June 3, 2015).

**342-0744 162453 Bassett, J. and M. Watson, “Dermal Sensitization study (closed-patch repeated insult) in guinea pigs with Chlorpyrifos Technical (Pyrinex),” Department of Toxicology, Ricerca, Inc., Painesville, OH, 3/31/98. Technical chlorpyrifos (97% purity) was administered to 20 Hartley guinea pigs for the induction phase at 50% concentration in peanut oil, 0.4 ml/site, administered to the shaved dorsal and lateral skin 3 times at weekly intervals. Challenge was 2 weeks after the last induction exposure, administered in 50% propylene glycol. Chlorpyrifos did not elicit a challenge response (i.e. is not a sensitizer). Positive control (DCNB) was effective. This study was considered as negative for sensitization and acceptable by DPR reviewers, D. E. Haskell and J. R. Sanborn (review of Dec. 2, 1998).

SUBCHRONIC STUDIES

Subchronic Oral toxicity, rat:

342-354 74494 Szabo, J. R., J. T. Young, and M. Grandjean, “Chlorpyrifos: 13-week dietary toxicity study in Fischer - 344 rats.” Lake Jackson Research Center [The Dow Chemical Co.], Freeport, Texas, 12/28/88. This study was submitted by Dow to contest the CDFA decision of a cholinesterase (ChE) NOEL at 0.05 mg/kg/day in the 2-year study, 345:072300. No comprehensive CDFA review of this subchronic study is necessary at this time, since the purpose of the 13-week study was to set dose levels for the cited 2-year study, which has already been accepted by CDFA. This subchronic study found statistically reduced plasma ChE levels ($p < 0.05$, two tailed) at day 44, but not at day 91. Investigators concluded findings at day 44 “not considered to be of toxicologic or biologic significance.” CDFA concludes that the findings are probably treatment effects, which however have no apparent toxicological consequence: the plasma ChE NOEL remains 0.05 mg/kg/day, but a practical NOAEL for ChE inhibition is 0.1 mg/kg/day. C. Aldous, 11/9/89.

Subchronic Oral toxicity, non-rodent: a supplementary 3-mo. dog study has been reviewed. No further non-rodent subchronic data are requested at this time.

342-306 063996 [Author appears to be McCollister, S. B.], “Results of 93-day dietary feeding studies of O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate in beagle hounds,” 1/15/64. This study pre-dates modern guidelines, and should be considered only for information on major symptoms of toxicity. Dogs were initially administered chlorpyrifos (98% purity) at 0, 200, 600, or 2000 ppm (report designates units of initial exposure as 0, 0.02, 0.06, and 0.2 percent in diet). There were 4 controls/sex, and 2/sex for each of the other groups. None of these treated dose

levels were sustainable, due to cholinergic symptoms such as “dilated and watery eyes, loose stools, vomiting, rough coats, labored breathing and tremors of the legs and head.” The 2000 ppm dogs were “essentially starving” as of treatment day 5, so that their diet was reduced to 0.006% (60 ppm) for the balance of the study. The dogs administered initially 600 ppm “were developing gross cholinergic symptoms,” and had diets reduced to 0.002% (20 ppm) after 16 days. Dogs originally administered 200 ppm were placed on control diet from day 45 onward. An additional group (N = 2/sex) was administered 200 ppm chlorpyrifos for about 45 days prior to sacrifice (designated as “Group B,” with estimated mean exposure of 3.4 mg/kg/day). Dogs were evaluated periodically for plasma and RBC cholinesterase (ChE), and brain acetylcholinesterase (AChE) was assessed at termination. Hematology, limited clinical chemistry, and terminal necropsy and histopathology were also recorded. These data were initially reviewed mainly to justify dose levels used in the chronic dog study (Record No. 036338). Small group sizes and altered dosing regimens limited the utility of this study. Group B 200 ppm dogs lost weight during their 45-day treatment, at a life stage when control dogs were still gaining weight. In particular one of the two Group B females lost 1.4 kg, and the other (which died shortly before scheduled sacrifice) lost 1.65 kg. The two Group B dogs surviving to termination and which had brain tissue assayed for AChE had brain AChE activities of about 50% of controls. The most relevant blood ChE data for these dogs was at 27 days of continuous treatment: at this time, the highly variable plasma ChE averaged about 10% of pre-exposure activity, and similarly variable RBC ChE activity was less than 20% of pre-exposure activity. Group A 200 ppm dogs had progressively diminishing plasma and RBC ChE activity over the time frame from 14 to 41 days of continuous exposure. When these dogs came off treatment, plasma ChE activity was visibly improving by 3 days, and was roughly 80% of pre-treatment levels by the 18th day off treatment. RBC ChE activity was slower to recover: with about 50% of pre-dosing activity between recovery days 18 and 32. RBC ChE activity was still below baseline at the last blood assay on recovery day 41. Brain AChE in these Group A 200 ppm dogs appeared to be in the normal range after 48 days of recovery. Dogs administered the medium dose (60 ppm for all but the first 5 study days) finished the study with plasma and RBC ChE activities at about 50% of pre-exposure values. At termination, males had brain AChE activity in the normal range, whereas females had implausibly low brain activities (i.e. lower than those observed in 200 ppm dogs after about 45 days of dosing). Dogs on the lowest sustained dose level (20 ppm) had plasma ChE activities of about 25% of pre-treatment levels, and RBC ChE activities of about 50% of pre-treatment levels. The 20 ppm males had normal brain AChE activity at termination, whereas one female had normal brain AChE activity, and one had about 40% of normal brain activity. In summary, although this study does not meet modern guidelines, had small group sizes and large variability in key responses, responses provide useful information on high dose effects to augment results from the later dog chronic studies. “One-liner” was re-written by Aldous on June 4, 2015 in support of risk assessment efforts in DPR.

Subchronic Inhalation toxicity, rat:

342-0967 284609 Newton, P. E., “A thirteen week nose-only inhalation toxicity study of chlorpyrifos technical (Pyrinex) in the rat,” Bio/dynamics Inc., East Millstone, NJ, 11/14/88, Project No. 88-8058. Fifteen F344 rats/sex/group were dosed by nose-only inhalation to chlorpyrifos vapors (Pyrinex Technical, 95% purity) at targeted concentrations of 0, 5, 10, and 20 ppb, respectively [6 hours/day, 5 days/week, for 13 weeks]. There were no treatment effects on clinical signs (in chamber or at detailed weekly examinations), or on body weight, food

consumption, hematology, clinical chemistry [other than possible plasma cholinesterase (ChE)]. Ophthalmology, necropsy observations, and histopathology findings were negative. Brain and RBC ChE activities were unaffected. The 20 ppb male plasma ChE activities were lower than any other contemporary groups and also lower than the limited pre-test ChE activities available. This reviewer considers that this represents a plausible treatment effect, with a NOEL of 10 ppb. NOEL for females = 20 ppb (no changes observed). This is a valid supplementary study (not a study design routinely expected under FIFRA requirements). See also the 1986 study: 342-0343 071389 (Corley et al.), which did **not** find any ChE effects at similar dose levels in nose-only vapor subchronic inhalation conditions like the present study. These equivocal, marginal plasma ChE findings are not designated as “possible adverse effects” under these circumstances. Aldous, June 3, 2015.

342-0967 284608. This is a brief report of corrections to 342-0967 284609, above. The cause of death had been erroneously coded for two rats in the original report. Survival was not dose-related in this study, and the corrections had no consequential impact on study interpretation.

Dermal toxicity, 21/28-day or 90-day:

342-0343 071391 Calhoun, L. L. and K. A. Johnson, “4-day dermal probe and 21-day dermal toxicity studies in Fischer 344 rats,” The Dow Chemical Company, Midland, MI, Sept. 1, 1988. Laboratory Study Nos. K-044793-085, K-044793-086. Chlorpyrifos, purity 100±0.1%, was applied in corn oil vehicle 6 hours/treatment to intact clipped dorsal skin (under gauze, secured by bandages) as indicated. Four female rats/sex/group were dosed by dermal application in corn oil at 0, 1, 10, 100, or 500 mg/kg/day for 4 consecutive days at 6 hours/treatment in a **probe study**. That study found that plasma cholinesterase was inhibited by 45%, 91%, and 97% at 10, 100, and 500 mg/kg/day, respectively. Also, RBC cholinesterase was inhibited by 16%, 49%, and 75% at respective dose levels. There were no other definitive findings in the probe study (which also assessed application site response, clinical signs, and body weight). The **primary** study was a 21-day dermal regimen, with dosing each weekday for a total of 15 exposures at 0, 0.1, 0.5, 1, or 5 mg/kg/day (N = 5/sex). Necropsy followed 2 consecutive treatment days in the final week. Investigators evaluated the parameters of the pilot study, plus a limited FOB, hematology, clinical chemistry, and histopathology. There were no definitive treatment effects in the primary study, hence the highest dose tested of 5 mg/kg/day is the NOEL for both sexes. This study is supplementary and not upgradeable (mainly because the dose range in the primary study was well below what the probe study showed to be supportable). Aldous, June 5, 2015.

CHRONIC STUDIES

Combined (chronic/oncogenicity), rat ** † (“possible adverse effect” based on non-oncogenicity findings in Record No. 153114, rat oncogenicity study)

**342-345 072300 Young, J. T., and M. Grandjean, “Chlorpyrifos: 2-year dietary chronic toxicity-oncogenicity study in Fischer-344 rats”. Dow Chemical Co., Freeport TX, 12/23/88. Chlorpyrifos (“AGR 214637”), 98.5%, in diet at 0, 0.05, 0.1, 1, and 10 mg/kg/day. 10/sex/dose designated for 1-year interim sacrifice: 50/sex/dose designated for 2-year duration. Cholinesterase (ChE) inhibition NOEL = 0.05 mg/kg/day (based on slight plasma ChE inhibition at 0.1 mg/kg/day in females). Acetylcholinesterase ChE inhibition NOAEL of 0.1 mg/kg/day is

nevertheless supportable, considering the issues discussed in the review for 354:074494. The NOEL for effects other than ChE inhibition was 0.1 mg/kg/day [based on very slight ($\leq 3\%$) but often statistically significant body weight decrease in 1 mg/kg/day males]. Body weights were statistically significantly reduced in 10 mg/kg/day males (7 to 9% throughout study). The “non-ChE effects” NOAEL was 1 mg/kg/day. Findings at 10 mg/kg/day were frequent perineal yellow staining in females, approximately 50% brain ChE inhibition in males and females, a slight increase in the degree of vacuolation of the adrenal zona fasciculata (males only), and a slight increase in diffuse retinal degeneration in 10 mg/kg/day females. None of these findings indicates possible adverse health effects (see review). ACCEPTABLE. C. Aldous, 4/21/89, 11/9/89 (see 354:074494). NOTE: Another rat study (see Record No. 153114 under AOncogenicity, Rat@ similarly identified retinal atrophy and cataracts at the highest dose tested (100 ppm in the latter case).

342-363 087917 (supplemental information to 342-345:072300). “Macroscopic postmortem examination of the eyes and associated structures in albino rats (Dow Method)”. (Refers to technique used at Freeport, TX, facility), method description dated 9/11/89. Methodology was presented in accordance with a CDFA request, which was made in the 4/21/89 CDFA review of the cited study. C. Aldous, 3/16/90.

342-250 and -251 036335-036337 McCollister, S. B., R. J. Kociba, P. J. Gehring, and C. G. Humiston, “Results of Two-Year Dietary Feeding Studies on DOWCO 179 in Rats” Dow Chemical, Midland, Michigan, 9/20/71. Chlorpyrifos, (presumed technical); 0, 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day in diet. NOEL cholinesterase enzyme inhibition = 0.1 mg/kg/day. NOEL for other systemic effects = 3.0 mg/kg/day (HDT). No oncogenicity observed. Incomplete, UNACCEPTABLE, and not upgradeable Too few animals, too much attrition due to disease (largely chronic murine pneumonia) & dose levels not justified and apparently below the MTD. C. Aldous, 1/28/86.

EPA 1-liner: [2-year feeding, rat, Dow Chemical Co, 9/20/71] Systemic NOEL 3.0 mg/kg/day (HDT); ChE NOEL = 0.1 mg/kg/day. Carcinogenic potential negative up to 3.0 mg/kg/day (HDT). Core grade, Supplementary.

342-044 031074 Published summary of 250/251:036335-036337.

342-013/053 031070 Summary of 250/251:036335-036337.

EPA 1-liner: [2-year feeding, rat, Dow Chemical Co, 9/20/71] Systemic NOEL 3.0 mg/kg/day (HDT); ChE NOEL = 0.1 mg/kg/day. Carcinogenic potential negative up to 3.0 mg/kg/day (HDT). Core grade, Supplementary.

342-044 031074 Published summary of 250/251:036335-036337.

342-013/053 031070 Summary of 250/251:036335-036337.

Chronic, dog **

**342-0252 036338-036339 McCollister, S. B., R. J. Kociba, P. J. Gehring, and C. G. Humiston, “Results of Two-Year Dietary Feeding Studies on DOWCO® 179 in Beagle Dogs,”

Dow Chemical, Midland, MI, 12/10/71. Chlorpyrifos (97.2% purity) was administered in diets at concentrations adjusted to provide 0, 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day. This study had two phases. In Phase A, there were 3/sex/group treated for 1 year, at which time 1/sex was necropsied. The remaining 2/sex were taken off treatment for 3 months prior to necropsy to evaluate recovery. In Phase B, 4/sex were dosed for 2 years at the above levels. Investigators assessed standard parameters of chronic studies. To assess cholinesterase (ChE) effects, plasma and RBC ChE activities were assayed 3 times pre-treatment and at 6 intervals during Phase A treatment. In Phase B, plasma and RBC ChE activities were assayed twice pre-treatment and at 8 intervals during treatment. Brain ChE was assessed at sacrifices of all dogs in both phases. Plasma ChE inhibition NOEL = 0.01 ppm, based on dose-related inhibition at 0.03 ppm and above. RBC ChE NOEL = 0.1 ppm, based on strong inhibition at 1.0 and 3.0 ppm compared to the same subjects at pre-treatment assessments. (See also Record No. 284915, which is a composite analysis of the RBC data from this study). Brain ChE activity at 3.0 mg/kg/day was reduced by an average of about 18%, with no evident sex difference in magnitude of response. There is a NOEL of 1.0 mg/kg/day for brain ChE. The NOEL for other effects, including behavioral observations, was the highest dose tested of 3.0 mg/kg/day. The study was designated as **acceptable** on 3/16/90, on receipt of details on preparation of treated food. Previous objections of CDFA to this study were (1) concerns that dosage range may not have adequately challenged the dogs, and (2) lack of reporting of ophthalmological examination data in the final report. These were addressed in submissions 306:063996 and 338:070883, respectively. This study was examined by C. Aldous on 1/29/86, 4/11/89, 3/16/90 (see also rebuttal response of 6/4/87 and minutes of meeting with Dow Chemical Co. representatives on 6/29/88). A final examination by Aldous on June 3, 2015 updated this summary and noted recent submission of the cited Record No. 284915 data. This study does not indicate an "adverse effect." ChE enzyme responses in this study are well-characterized and consistent with results of other rat dietary studies such as the rat subchronic, developmental toxicity, and reproductive effects studies.

342-363 087918 (Addendum to 342-252:036338, combined dog study). Submission contains mean body weights/sex and average food consumption for a 6-week period. At the end of the 6-week period, it was determined that 100 ppm in diet corresponded closely to 3.0 mg/kg/day in either sex. From that time on, diets were prepared at fixed levels of 100, 33, 3.3, 1.0, and 0.33 ppm by serial dilutions of diets. These data permit an upgrade of the 1971 dog study to ACCEPTABLE status. Aldous, 3/16/90.

342-0969 270309 (Supplementary to Document No. 342-0252, Record Nos. 036338-036339), Authors of the re-analysis are Mattsson, J. L., L. Holden, D. L. Eisenbrandt, and J. E. Gibson. "Reanalysis with optimized power of red blood cell acetylcholinesterase activity from a 1-year dietary treatment of dogs to chlorpyrifos." The date of the re-analysis was 9/22/2000. Study ID: GHC-5127. Chlorpyrifos (97.2% purity) in the dog chronic study was administered in diets at concentrations adjusted to provide 0, 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day. That study had two phases at the above dose levels, which were comparable in design, so that parallel results could properly be considered together. The present analysis was confined to RBC acetylcholinesterase (AChE) inhibition analysis. Four figures show RBC AChE activities by phase and sex consistent with tabular summary data in Record No. 036338. These figures show marked inhibition of RBC AChE activity at 1.0 and 3.0 mg/kg/day, whereas AChE activities of other groups tended to

cluster together at any given time point. Individual pre-treatment AChE activities had more influence on subsequent treatment-phase activities than did possible treatment group effects, except at the two highest dose levels. When investigators normalized the baseline for each group pre-treatment mean, combining data for both sexes in both phases at assay intervals during the first year gave N = 14. A depiction of inter-group differences on this basis found no meaningful differences between control and treatment groups through 0.1 mg/kg/day. When all assays during the first year of treatment were considered together for each group, activity of the 1.0 mg/kg/day group was nearly 50% below baseline, and the 3.0 mg/kg/day group activity was 80% below baseline, whereas all other groups remained within about 4% of baseline. Collectively, these amalgamated data support a NOEL of 0.1 mg/kg/day for RBC AChE. Aldous, June 2, 2015.

342-273 056902 (Tab 3) EPA Office of Pesticide Programs, Toxicology Branch review of study 252:036338-036339. The review was submitted on Oct. 10, 1985 as OPP Toxicology Branch Document #004712. The review classified the study as “Core Minimum Data”.

EPA 1-liner: [2-year feeding - dog; Dow Chem. Co.; 12/10/71] Systemic NOEL = > 3.0 mg/kg/day (HDT); Plasma ChE NOEL = 0.01 mg/kg/day; Plasma ChE LEL = 0.10 mg/kg; RBC ChE NOEL = 0.10 mg/kg/day; RBC ChE LEL = 1.0 mg/kg; Brain ChE NOEL = 1.0 mg/kg/day; Brain ChE LEL = 3 mg/kg; Core grade, supplementary [note upgrade to “core minimum” status, indicated in 273:042783].

342-338 070881-070882 are dietary analyses and analytical methods descriptions. These data were evaluated with respect to study 252:036338 in the 4/11/89 CDFA review.

342-338 070883 is a supplement to the original 2-year dog feeding study report. Supplement included ophthalmology data. These data had been submitted to EPA in 1985. These data were evaluated with respect to study 252:036338 in the 4/11/89 CDFA review.

342-044 031073 Published summary of 252:036338.

342-013/053 031070 Summaries of 252:036338-36339.

Oncogenicity, rat (see “Combined, Rat” above)

****342-692 153114** Crown, S., “Pyrinex technical oncogenicity study in the rat”, Life Science Research Israel, Ltd., July 12, 1990. Laboratory Study # MAK/095/PYR. Pyrinex (chlorpyrifos), 96.1% purity, was administered in diet to 60 F344 rats/sex/group at 0.2, 5, and 100 ppm. There were two control groups (with and without corn oil mixing supplement), each composed of 60/sex/group. Treatment was for 2 yr, except that 5/sex/group were sacrificed at wk 50 for brain cholinesterase (ChE) assays. ChE enzyme inhibition NOEL = 0.2 ppm (inhibition of plasma ChE at 5 ppm). NOEL for non-ChE-related changes = 5 ppm. No definitive cholinergic signs were evident at any dose level. Findings at 100 ppm included modest body weight decrements and over 50% brain ChE inhibition in both sexes, and an increase over baseline incidences of diffuse retinal atrophy and cataracts in 100 ppm females. The latter findings are “**possible adverse effects**” in an **acceptable** oncogenicity study. Aldous, 8/28/97.

Oncogenicity, mouse **

342-693 153115 Gur, E., "Pyrinex technical oncogenicity study in the mouse", Life Science Research Israel, Ltd., 10/15/92. Laboratory Study # MAK/106/PYR. Fifty-nine CD-1 mice/sex/group were dosed for 79 weeks with Pyrinex technical (chlorpyrifos) in diet at 0, 5, 50, or 250 ppm. An additional 5/sex/group were killed at week 42 for cholinesterase (ChE) evaluation. There was no ChE NOEL in the tested dosage range (dose-related inhibition of plasma ChE in both sexes at weeks 42 and 78). Brain ChE was modestly reduced at 50 ppm and greatly reduced at 250 ppm (residual activity about 20% or less in both sexes and both sampling intervals). RBC ChE was reduced at 250 ppm only. There were no definitive cholinergic signs at any dose. NOEL for other effects was 5 ppm (males displayed excessive lacrimation, opaque eyes, and hair loss around eyes: all plausibly related to contact irritability of test article with resultant scratching). High dose findings, in addition to signs consistent with local irritation, included hepatocyte vacuolation and cystic dilatation of bulbourethral glands (males), and alveolar macrophage accumulation in lungs (females). Male body weights and food consumption were decreased at 250 ppm, and water consumption was sharply reduced in both sexes at that dose level. Survival of high dose males was remarkably higher than other groups. This is an **acceptable oncogenicity study with no adverse chronic effects. Aldous, 8/22/97.

**342-253 036340 Warner, S. D., C. G. Gerbig, R. J. Strebing, and J. A. Molello, "Results of a two-year toxicity and oncogenic study of Chlorpyrifos administered to CD-1 mice in the diet," Dow Chemical Toxicology Laboratory, Indianapolis, Indiana, 3/4/80. Chlorpyrifos, Ref. No. 1-500-2: 99.6% purity at 0, 0.5, 5.0, and 15.0 ppm in diet. NOEL = 15 ppm (no toxicity). No oncogenicity. ACCEPTABLE, based on re-reading of blood smears by S. D. Warner, D.V.M., Ph.D. (data in CDFR record 315:065762) answering a question by CDFR regarding possible effects on lymphocytes, (see 5/29/87 CDFR review). (Other concerns which CDFR had on this report were addressed in the 5/29/87 CDFR review). C. Aldous, 1/31/86, 5/29/87, 4/12/89.

342-273 042782 (Tab #4) Supplemental to 253:36340. Davies, D. B., J. T. Tollett, and L. G. Lomax, "Chlorpyrifos: A Four-Week Dietary Study in CD-1 Mice," Dow Chemical, Midland, MI. Dietary administration of 0 or 15 ppm chlorpyrifos (95.7% purity) to CD-1 mice. 4 week study with body weights slightly reduced and plasma and serum ChE levels statistically significantly reduced (see especially, Table 13). This study supports dose level selection for the oncogenicity study (such as 253:036340, above). After 4 weeks, treated mice had about 10% of control plasma cholinesterase (ChE) activity, and about 50% of RBC ChE activity. Brain AChE activity was statistically reduced in treated females and statistically elevated in treated males: magnitudes were small in both cases and appear to have been incidental. Examined 11/24/86 and again on 6/4/15 by C. Aldous. No written review was required or performed.

EPA 1-liner: [2-Year oncogenic - mice; Dow Chemical Co.; 3/04/80]: Systemic and oncogenic NOEL > 15 ppm (HDT). Core grade, minimum.

342-290:050623 (Rebuttal/Additional data to 253:36340) "Results of a Two-Year Toxicity and Oncogenic Study of Chlorpyrifos Administered to CD-1 Mice in the Diet". Dow Chemical Toxicology Laboratory, 3/4/80. New information consists of individual data for blood smear exams, clinical observation and animal disposition, and gross and histopathology. Reviewer (Aldous) examined previously submitted chemical analyses of test material used in this and in

one other study, and included evaluation in 5/29/87 review. No adverse effects noted. Study not acceptable, but possibly upgradeable. C. Aldous, 5/29/87.

342-013/053 031071 Summary only of 253:036340.

GENOTOXICITY

Bacterial reverse mutation assay ** (see after In vitro mammalian cell assay section for summary statement)

342-255 036348 Simmon, V. F., A. D. Mitchell, and T. A. Jorgenson, "Evaluation of Selected Pesticides as Chemical Mutagens, in Vitro and in Vivo Studies," (brief summary) SRI, 1977; Salmonella and E. coli. UNACCEPTABLE with no adverse effect reported. Salmonella, 4 strains (no TA98), were tested with and without activation at 0, 1, 5, 10, 50, 100, 500 and 1000 µg/plate and with Escherichia coli at the same concentrations. Chlorpyrifos, 98.8%. No evidence of a cytotoxic concentration or rationale for maximum concentration used. No repeat trial, no individual plate counts if more than one was made. Not upgradeable. J. Gee, 2/13/86.

342-273 042784 Bruce, R. J. and J. A. Zempel, "Chlorpyrifos: Evaluation in the Ames' Salmonella/Mammalian-Microsome Mutagenicity Assay," Dow Chemical, Freeport, Texas, 1986; Salmonella. Chlorpyrifos (95.7%) tested in strains TA1535, TA1537, TA98 and TA100 at 0, 1, 3.16, 10, 31.6 and 100 µg/plate; with and without rat liver activation; 30 min pre-incubation before plating, triplicate plates, one trial, no evidence for increased reversion rate. UNACCEPTABLE. Report states that a precipitate formed at 100 µg/plate. The earlier study did not mention this. J. Gee, 7/30/86.

342-419 116728. Supplement to 042784. Contains individual plate counts and a revised table of contents. No change in the study status. No worksheet. Kellner and Gee, 7/9/93.

Mutagenicity: In vitro mammalian cell assay **

**342-255 036351 Mendrala, A. L., "Evaluation of Chlorpyrifos in the Chinese Hamster Ovary Cell-Hypoxanthine (Guanine) Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay," Dow Chemical, Midland, MI, Sept. 3, 1985. Chlorpyrifos, 95.7% purity, was tested at 0, 10, 20, 25, 30, 40 or 50 µM with and without activation for 4 hours. Positive control was 3 mM EMS. There were 5 dishes per treatment, in a single trial. A precipitate formed at 30 µM and above. Survival percentages (relative to 0 µM control) at chlorpyrifos levels of 10, 20, 25, 30, 40 or 50 were 92, 31, 23, 16, 9, and 7%, respectively. Testing thus bracketed practical limits based on both solubility and cytotoxicity. There was no increase in mutation frequency reported for chlorpyrifos in any single trial. Positive control mutation frequency was about 100x above background. Initially, results were considered to be negative for chlorpyrifos mutagenicity, however study was designated as unacceptable, based on lack of a confirming trial (see original review by J. Gee, 2/13/86). Current guidelines (OPPTS 870.5300, page 7) do not routinely require a repeat this assay after a negative response. Consistent with contemporary guidelines, study should be re-classified as acceptable, with no adverse effects. Aldous, June 5, 2015.

342-291 [No Record No., second “Mutagenicity” tab in volume]. Rebuttal comments ref 255:036351. CDFA conclusion was study still UNACCEPTABLE: major concern remaining is lack of a confirmatory test for a negative result. (J. Gee, 6/5/87).

342-291 057655 A table entitled “Analytical determination of stability of Chlorpyrifos in DMSO” in support of 255:036351, above. (Submitted as part of rebuttal document of 12/1/86).

***SUMMARY: The 1977 SRI study (#036348), using four strains of Salmonella (but not TA98) at 0 to 1000 µg/plate, was negative for increased reversion. Also, the CHO/HGPRT study on file showed negative results. EPA accepted this CHO study (#036351) although CDFA review found it unacceptable because there was no repeat. Considering all of these studies, with no one alone being acceptable, and that #042784 is a repeat of #036348 -- the deficiency for which each was rejected separately -- the 842 data gap is considered filled.

Mutagenicity: In vivo cytogenetics **

342-419 116722 “Evaluation of Chlorpyrifos in an In Vitro Chromosomal Aberration Assay Utilizing Rat Lymphocytes”, (Linscombe, V., Mensik D. and Clem, B., Dow Chemical Company, Lab Project Study ID: K-044793-092, 1/29/92). Chlorpyrifos, purity of 98.6%, was evaluated for clastogenic potential using rat lymphocytes treated for 4 hours with concentrations of 0 (DMSO), 5, 16.7, 50, 167.7, 500, 1667.0 or 5000 mg/ml (Assay 1) and 0, 5.0, 16.7, 50.0 and 167.0 mg/ml (Assay 2) with and without S-9 metabolic activation. Cultures were harvested 24 hours after treatment in Assay 1 and 24 and 48 hours after treatment in Assay 2. **No Adverse Effects: No increase in chromosomal aberrations at the highest scorable dose levels of 167 mg/ml (without S-9) and 50 mg/ml (with S-9). ACCEPTABLE. (Kishiyama, Kellner and Gee, 7/1/93).

342-739 161321 Exact duplicate of 342-419 116722 (above). This was submitted in a volume which contained primarily product chemistry data. Aldous, 11/12/98.

342-363 087919 McClintock, M. L., and B. B. Gollapudi, “Evaluation of Chlorpyrifos in the Bone Marrow Micronucleus Test.” (Dow, TXT: K-044793-067A, 9/22/89). Chlorpyrifos, lot AGR 214637, 97.9%; tested with CD-1 (ICR) BR mice, with sacrifices of 5/sex/group at 24, 48 or 72 hours after a single oral gavage dosing of 0 (corn oil) or 90 mg/kg b. wt. stated to be 80% of the LD₅₀; cyclophosphamide as positive control; no mortalities but decrease in body weights in the treatment groups; no evidence of micronuclei formation and no clear effect on PCE/NCE. UNACCEPTABLE (only one dose level). (Gee, 3/12/90)

342-255 036350 Gollapudi, B. B., V. A. Linscombe, and J. E. Wilkerson, “Evaluation of Chlorpyrifos in the Mouse Bone Marrow Micronucleus Test,” Dow Chemical, Freeport, Texas, 1985; Mouse micronucleus test. UNACCEPTABLE with no adverse effect. Chlorpyrifos, 95.7%, was given by oral gavage to 5/sex/group at 0, 7, 22, or 70 mg/kg with sacrifices at 24 and 48 hours. No statistically significant increase in micronuclei in PCE's is reported; % PCE marginally effected in females only at 48 hours being 63 as compared with 76 for the vehicle control. This is suggestive that a higher dose and/or a longer sampling time should have been included even at the risk of losing some of the animals. In the Appendix data show that survival at 100 mg/kg would be adequate for the assay. Also, no clinical signs were observed. The high

dose reportedly was based on 60% of the LD50 of approximately 111 mg/kg. Guidelines and the meaningfulness of the test call for some signs than a toxic dose was reached, either the MTD for the animal or cytotoxicity to the bone marrow. The only death was in female vehicle control. No data on micronucleated normochromatic erythrocytes are included. Because positive effects have been reported in gene conversion and DNA repair, an adequate test in this test area is needed. Not upgradeable. J. Gee, 2/13/86.

NOTE: EPA considers this study as acceptable, according to the EPA response to CDFA data gap status issues on chlorpyrifos, dated 1/17/89. Aldous, 12/4/89.

342-291 [No Record number, first "Mutagenicity" tab in volume]. Rebuttal comments ref 255:036350. CDFA conclusion was study still UNACCEPTABLE: major concerns remaining are inadequate justification of treatment levels, and lack of a 72 hr sacrifice time. J. Gee, 6/5/87.

Mutagenicity: DNA Damage (not a normally required test category) ** †

342-255 036349 Simmon, V. F., A. D. Mitchell, and T. A. Jorgenson, "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies," [Segment on mammalian *in vitro* unscheduled DNA synthesis assays] SRI, 1977; UDS in WI-38. UNACCEPTABLE but upgradeable with no adverse effect reported. Chlorpyrifos, 98.8%. WI-38, human embryonic lung fibroblasts, were exposed with and without activation (rat liver) to 0, 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} , and 10^{-3} with six cultures -S9 and 3 +S9. DPM/ μ g DNA is reported with no change in the DPM with increasing concentrations. DNA was extracted from the cells by a standard method and an aliquot used to determine the amount of DNA and another portion used to determine the incorporation of tritiated thymidine by liquid scintillation counting as a measure of DNA repair in response to damage by the test article. Missing information on how the CPM were converted to DPM, the quantity of DNA recovered per culture, the passage number of the WI-38, and the rationale for the selection of the concentrations used - whether solubility or cytotoxicity. CDFA review 2-13-86 J. Gee.

342-255 036347 Simmon, V. F., A. D. Mitchell, and T. A. Jorgenson, "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies --Microbiological Assays" (summary report), SRI, 1977; *Saccharomyces cerevisiae* D₃. UNACCEPTABLE with a positive effect reported. Mitotic recombination-gene conversion in yeast exposed to a 5% concentration for 4 hours, with and without metabolic activation. The test was repeated. No individual data. Because of the lack of data, the significance of the effect cannot be evaluated but the possible genotoxic effect must be noted. Upgradeable. J. Gee, 2/13/86.

342-255 042609 Simmon, V. F., A. D. Mitchell, and T. A. Jorgenson, "Evaluation of Selected Pesticides As Chemical Mutagens, In Vitro and In Vivo Studies -Microbiological Assays" (summary), SRI, 1977; *Escherichia coli* and *Bacillus subtilis* [found under Tab 12, pg. 20]. UNACCEPTABLE with a positive adverse effect reported. Chlorpyrifos, 98.8% purity, at 2.5 μ g/disc, was tested with *E. coli* W3110 and p3478 and with *B. subtilis* H17 and M45. No activation was included and the test reportedly was repeated 3 times. The comparable zones of inhibition between the strains indicated a larger zone for the repair defective strains. Only one value for each strain is reported. If the full report were submitted, it is possible that the effect could be evaluated for significance. Since no activation was included, the study is not upgradeable. J. Gee, 2/13/86.

**342-273 042785 Mendrala, A. L. and M. D. Dryzga, "Evaluation of Chlorpyrifos in the Rat Hepatocyte Unscheduled DNA Synthesis (UDS) Assay," Dow Chemical, Midland, MI, 1986; Chlorpyrifos (95.7%); primary rat hepatocytes tested for unscheduled DNA synthesis at 10^{-6} , 3.13×10^{-6} , $x 10^{-5}$, 3.16×10^{-5} and 1×10^{-4} M; triplicate cultures in a single trial; no evidence of UDS; toxicity at the highest concentration. Acceptable. J. Gee, 7/30/86.

SUMMARY: The positive findings in the two microbial studies are somewhat related. The B. subtilis test compares the response of rec^{-} (recombination defective) with wild type organisms. The rec^{-} strain is not as competent to repair damage and hence shows a greater inhibition of growth from lethality due to DNA damage. The test in Saccharomyces also measures recombination-type events in competent organisms and the increase in these events confirms the DNA damage. The complete versions of these two reports are needed to assess their significance. The two tests in mammalian cells measure a different repair event (excision repair) with repair replication occurring to fill the DNA gap following removal of damaged bases by excision using different enzymes. The positive findings in the microbial tests cannot be dismissed without more information about the bacterial studies.

REPRODUCTIVE TOXICITY, RAT **

342-399 097570 "Chlorpyrifos: Two-generation dietary reproduction study in Sprague-Dawley rats", (W. J. Breslin, A. B. Liberacki, D. A. Dittenber, K. A. Brzak, and J. F. Quast). The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI., Study ID: K-044793-088, 6/5/91). Chlorpyrifos, (technical grade Dursban F insecticide, AGR 273801), 98.5% purity, was fed in the diet to 30 Sprague-Dawley rats/sex/group through 2 generations with 1 litter per generation. Concentrations were adjusted as needed to achieve exposures of 0, 0.1, 1.0, and 5.0 mg/kg/day. Treatment began approximately 10 and 12 weeks prior to breeding for the F0 and F1 adults, respectively. Cholinesterase (ChE) inhibition NOEL = 0.1 mg/kg/day (Plasma and RBC ChE inhibition at 1.0 and 5.0 mg/kg/day). Parental NOEL = 1.0 mg/kg/day (increased degree of vacuolation in zona fasciculata, especially in males; altered tinctorial properties in this tissue in females). Reproductive NOEL = 1.0 mg/kg/day (slightly reduced pup weights and slightly reduced pup survival at 5.0 mg/kg/day). There were no clinical signs specifically indicating ChE inhibition. The reproductive findings at 5 mg/kg/day do not warrant a "possible adverse effects" designation, since brain ChE levels were very markedly depressed at that dose level, and all observed reproductive effects appeared to be due to failure of dams to nurture pups which were otherwise normal. **ACCEPTABLE. (Green and Aldous, 5/11/92).

342-685 152365 Exact duplicate of 342-399 097570.

342-374 090493 Interim report for Record No. 097570, above.

342-686 152368 Breslin, W. J., A. B. Liberacki, D. A. Dittenber, and J. F. Quast. "Evaluation of the developmental and reproductive toxicity of chlorpyrifos in the rat". *Fundam. Appl. Toxicol.* **29**:119-130 (1996). This is a published summary of major findings of two accepted studies: the reproduction study above (342-399 097570) and the rat teratology study (342-254

036344). Since the abstract was consistent with DPR 1-liner conclusions for the two studies, this publication was not independently reviewed. Aldous, 7/31/97.

342-254 036341 “Three Generation Reproduction and Teratology Study in the Rat Following Prolonged Dietary Exposure to Dursban, O,O-Diethyl O-3,5,6-Trichloro-2-Pyridyl Phosphorothioate,” Dow Chemical, Zionsville, Indiana, 8/20/71. Chlorpyrifos, purity and grade not specified. Doses for the main portion of the reproduction study were 0, 0.1, 0.3, and 1.0 mg/kg/day in diet. ChE inhibition NOEL= 0.3 mg/kg/day. General adult toxicity NOEL = 1.0 mg/kg/day (HDT). Reproductive NOEL = 0.3 mg/kg/day (slightly increased pup mortality in first 5 days post-partum) UNACCEPTABLE, incomplete, not upgradeable (more definitive follow-up study is 254:036343). C. Aldous, 1/31/86.

(An additional copy of 036341 is found in Document No. 342-685, Tab 49 (no record #). EPA 1-liner: [3-Generation reproduction/teratology - rat; Dow Chem. Co.; 8/20/71] Reproduction NOEL>1.0 mg/kg/day (HDT); Teratogenic NOEL = inconclusive. ChE NOEL=0.1 mg/kg Core grade, minimum

342-254 036343 Dietz, F. K., D. C. Mensik, C. A. Hinze, B. L., Rachunek, and H. W. Taylor, “Dursban Insecticide: Assessment of Neonatal Survival In A Two-Generation Reproduction Study In Rats,” Dow Chemical, Freeport, Texas, 7/83. Chlorpyrifos, technical; 0, 0.5, 0.8, and 1.2 mg/kg/day (dietary). Parental toxicity NOEL = reproductive toxicity NOEL = highest dose tested = 1.2 mg/kg/day. UNACCEPTABLE, incomplete, upgradeability unlikely (highest dose level not demonstrably toxic, and no justification offered for dosage selection). C. Aldous 2/7/86.

EPA 1-liner: [Two generation repro - rat; Dow Chem.: 7/83] Reproductive NOEL > 1.2 mg/kg/day (HDT); Systemic NOEL = 0.8 mg/kg; Systemic LEL= 1.2 mg/kg (decreased weight gain); Core grade, supplementary.

342-681 152366 Exact duplicate of 254 036343, above.

342-291: [No Record #, Tab = “Reproduction”] Rebuttal comments ref. rat reproduction studies 254:036341 and 254:036343. Registrant noted that CDFA should consider both reproduction studies together, considering additionally rat chronic data. Registrant suggested that plasma and RBC ChE inhibition data support adequacy of dose. CDFA response: Doses are not justified in terms of parental toxicity, notwithstanding enzyme inhibition effects. Chronic studies are imperfect surrogate studies for evaluation of microscopic changes due to test article, since in chronic studies there is no evaluation of effects which carry over the generations. No change in status of studies. C. Aldous, 6/2/87.

342-686 152367 James, P., A. Stubbs, C. A. Parker, J. M. Offer, A. Anderson, “The effect of Pyrinex (chlorpyrifos) on reproductive function of two generations in the rat”, Huntingdon Research Centre, Ltd., 4/22/88. HRC Report # MBS 29/881452. Cr1:CD®(SD)BR rats received diets containing 0, 2, 10, or 50 ppm chlorpyrifos (95% purity) in diets over 2 generations (1 litter per generation). Parental rats numbered 28/sex/group in the F0 generation, and 24/sex/group in the F1 generation. Protocol was that of a standard reproduction study, with a few pre-weaning developmental evaluations added (surface righting, air righting, and startle responses; and pupil

reflex). There were **no definitive treatment-related effects** (report attributes 3 high dose deaths to treatment, however there were deaths in other groups and no evident unique symptoms in high dose decedents). Study is **not acceptable** as presented (report evidently contains 401 pages, but only pp. 1-228 are present, “confidentiality” stamps cover much of the text, more definitive high dose justification would be needed, and histopathology of parental rats is needed if this study is to be upgraded). Aldous, 8/22/97.

DEVELOPMENTAL TOXICITY

Rat Developmental Toxicity **

**342-254 036344 Ouellette, J. H., D. A. Dittenber, P. M. Kloes, and J. A. John, “Chlorpyrifos: Oral Teratology Study in Fischer 344 Rats,” Toxicology Research Lab., Dow Chemical USA, Midland, MI, 7/5/83. Chlorpyrifos, 96.6%. 0, 0.1, 3.0, and 15 mg/kg/day (gavage). Maternal NOEL (excluding cholinesterase (ChE) inhibition) = 3.0 mg/kg/day (cholinergic effects). Maternal ChE inhibition NOEL = 0.1 mg/kg/day (inhibition of plasma and RBC ChE). Developmental toxicity NOEL = 15 mg/kg/day (HDT). ACCEPTABLE due to submission of supplementary information. See CDFA Rebuttal comments, C. Aldous, 6/1/87. (Study had been classified unacceptable in previous review by C. Aldous 2-10-86). C. Aldous, 6/1/87. EPA 1-liner: [Teratology - rat; Toxicology. Research Lab; 7/5/83] Teratogenic and fetotoxic NOEL > 15 mg/kg/day (HDT); Maternal NOEL = 0.1 mg/kg; Maternal LEL = 3.0 (ChE inhibition) Core grade, minimum.

342-683 152360 (exact duplicate of 342-254 036344, above).

342-291 050624 (Rebuttal by Ouellette *et al.* to primary study 254:036344). Considered in 6/1/87 review of primary study, 254:036344, above.

342-291 050625 (Pilot study to primary study 254:036344). Ouellette, J. H., D. A. Dittenber, R. J. Kociba, and J. A. John, “Chlorpyrifos: Oral teratology probe study in rats”. Toxicology Research Lab, Dow, 1/4/83.

Chlorpyrifos, 96.6%. 0, 3, 10, and 30 mg/kg/day by gavage in cottonseed oil. Study demonstrates that 30 mg/kg/day is severely toxic to dams: maternal deaths, typical cholinergic signs, high number of resorptions. Slightly matted haircoat and slight enlargement of adrenals were observed at 15 mg/kg/day. This pilot study clearly substantiates the adequacy of the dosage range selected for the primary study, 254:036344. C. Aldous, 6/1/87.

342-695 153117 Rubin, Y., N. Gal, T. Waner, and A. Nyska, “Pyrinex teratogenicity study in the rat”, Makhteshim-Agan of North America Inc., 7/15/87. Laboratory Study #MAK/101/PYR. At least 21 pregnant CD rats/group were dosed with Pyrinex Technical (chlorpyrifos), purity 96.1% by gavage in corn oil on days 6-15 p.c. at 0, 0.5, 2.5, or 15 mg/kg/day. No maternal ChE NOEL was identified (dose-related plasma ChE inhibition at all dose levels at day 15 p.c., with restoration of normal ChE activity in all but high dose dams by p.c. day 20. Maternal functional NOEL = 2.5 mg/kg/day (tremors in 3/21 dams, transient food consumption reduction, modest but consistent body weight decrement). Developmental NOEL = 2.5 mg/kg/day (slight increase in early resorptions). **No adverse reproductive effect at dose levels sufficient to elicit cholinergic responses. Acceptable. Aldous; May 1, 1997.

342-683 152361 Exact duplicate of 342-695 153117, above.

342-681 152354 Muto, M. A., F. Lobelle, J. H. Bidanset, and J. N. D. Wurpel, "Embryotoxicity and neurotoxicity in rats associated with prenatal exposure to Dursban", *Veterinary and Human Toxicology* 34, 498-501 (1992). Investigators from the Department of Pharmaceutical Sciences, St. John's University, Jamaica, NY. Test article was a formulation of 1% chlorpyrifos, 6% xylene, and 93% water. Suspensions were diluted to an unspecified dosing volume with saline. Dosing was ip, either on days 0-7 or on days 7-21 at dose levels of 0, 0.03, 0.1, or 0.3 mg/kg/day of chlorpyrifos. In most cases, there were 8 pregnant rats (strain unspecified) per dose for each treatment time period. Dams were allowed to litter, then pups were evaluated for "general viability, body weight and physical characteristics". Selected pups were evaluated for "neurotoxicity" on a rotarod on day 16. The same day, pups were evaluated for motor behavior (subjective open field observation) and for righting behavior on an inclined screen. An additional study evaluated the neurotoxicity and behavioral tests following exposures of 0.1 or 0.3 mg (presumably ip) as single doses on day 3, 10, or 12 postpartum, or as multiple doses on days 6-10 postpartum. Investigators claimed that treatment caused increased embryoletality following dosing on gestation days 0-7 and gestation days 7-21. Since the highest embryoletality was in the lowest dose group treated on gestation days 0-7 (77% lethality), these data are of questionable value. Incidences of "physical abnormalities" were reportedly highest in 0.1 and 0.3 mg/kg/day groups (66 and 55%, respectively), among litters treated on gestation days 0-7. No corresponding control data were presented. Rotarod performance was reported to be impaired in pups dosed at 0.3 mg/kg on days 3, 10, and 12, and in offspring of dams dosed with 0.3 mg/kg on days 7-21, and in offspring of dams dosed with 0.03, 0.1, or 0.3 mg/kg on days 0-7. These data are suspect because differences between mean values at any treatment time dwarfed differences between dose groups at individual treatment times, even though all pups were evaluated at day 16. The study is unacceptable (in addition to deficiencies noted above, test article does not represent either the a.i. or any end use product; the route (ip) is not a plausible route of human exposure; the conclusions are speculative, evidenced by discussion of possible delayed distal neuropathy, while ignoring a valid 1986 subchronic hen neurotoxicity study, which would have been available through "freedom of information" provisions long before the time of this publication; and the presentation of the article shows that it could not have gone through a meaningful review, indicated by the above deficiencies, and by misspellings (the term "access" when "assess" was meant) and by failures to provide control data in figures or to provide numerical counts for types of purported treatment-caused malformations. No more information is requested of this paper. Aldous, 9/3/97.

342-681 152355 Nimphius, M. J. (M.S. dissertation under direction of graduate advisor J. H. Bidanset at St. John's University College of Pharmacy and Allied Health Professions, New York). "The effects of chlorpyrifos and xylene on embryonal and fetal development in the rat" (approval date: 9/13/95). Sprague-Dawley rats were dosed subcutaneously with 0, 0.3, 3, or 10 mg/kg/day chlorpyrifos (analytical grade, 99% purity) on days 1-7 of gestation (typically 8/dose/group), then sacrificed on gestation day 19 or 20. Other rats received xylene or chlorpyrifos/xylene s.c. on the same schedule. Parameters examined were resorptions, weights and lengths of fetuses, and external malformations. None of these showed biologically meaningful changes. This study is unacceptable (it does not conform to any FIFRA study

design: route is not relevant to plausible human exposure, timing of dosing is not useful for evaluation of malformations, fetal examinations were only for grossly evident changes, group sizes were too small, and sacrifices were not done on a fixed gestation day). The study does not make a significant contribution to chlorpyrifos hazard assessment. Aldous, 9/3/97.

[Rat Developmental Toxicity Studies: Chlorpyrifos Metabolites]

342-684 152362 Hanley, T. R., G. J. Zielke, and L. G. Lomax, “3,5,6-Trichloro-2-pyridinol: oral teratology study in Fischer 344 rats”, The Dow Chemical Co., Midland, MI, 7/23/87. Laboratory Study #: K-038278-011. Groups of 32-34 mated Fischer 344 rats were dosed with 0, 50, 100, or 150 mg/kg/day 3,5,6-trichloro-2-pyridinol (TCP, 99.7% purity) by gavage in 4 ml/kg Methocel on days 6-15 of gestation in a standard teratology study. Maternal NOEL = 50 mg/kg/day (minor body weight gain decrements). Developmental NOEL = 150 mg/kg/day (HDT). An acceptable study of a major metabolite of chlorpyrifos, with no adverse effect indicated. Aldous, 7/31/97.

Rabbit Developmental Toxicity ** (No adverse effects for technical chlorpyrifos, however high doses of a metabolite caused developmental toxicity)

342-694 153116 Rubin, Y., A. Nyska, and T. Waner, “Pyrinex teratogenicity study in the rabbit”, Life Science Research Israel Ltd., 7/15/87. Laboratory Study # MAK/103/PYR. At least 14 HY/CR (a NZW variety) rabbits per group were dosed by gavage in corn oil with chlorpyrifos (Pyrinex Technical, purity 96.1%) on days 7-19 p.c. at 0, 1, 9, 81, or 140 mg/kg/day. Maternal NOEL = 81 mg/kg/day (body weight gain decrement during treatment period). Developmental NOEL = 81 mg/kg/day [reduced crown/rump length, reduced fetal weight, ossification delays (indicated by non-ossification of fifth sternebra and/or xiphisternum)]. No adverse effects are indicated. For comparison, the pilot study had found 100% lethality in does at 270 mg/kg/day. **Acceptable. Aldous, 4/29/97.

342-685 152364 Exact duplicate of 342-694 153116, above.

[Rabbit Developmental Toxicity Studies: Chlorpyrifos Metabolites]

342-684 152363 Hanley, T. R., G. J. Zielke, and L. G. Lomax, “3,5,6-Trichloro-2-pyridinol: oral teratology study in New Zealand White rabbits”, The Dow Chemical Co., Midland, MI, 7/23/87. Laboratory Study #: K-038278-015. Sixteen does/group were dosed with 0, 25, 100, or 250 mg/kg/day 3,5,6-trichloro-2-pyridinol (TCP, purity 99.7%) by gavage in aqueous 0.5% Methocel on gestation days 7-19 in a teratology study. Maternal NOEL = 100 mg/kg/day (minor maternal body weight decrement during treatment). Developmental NOEL = 25 mg/kg/day (hydrocephaly and dilated cerebral ventricles). The latter observations were not statistically significantly increased in either of the two higher dose groups compared to concurrent controls, however historical background incidences were very low (compare hydrocephaly litter incidences of 2/13 and 3/13 at 100 and 250 mg/kg/day, respectively, to a historical incidence of 1/839 litters). These findings indicate a **possible adverse effect**. For perspective, 100 mg/kg/day of TCP is the molar equivalent to 66% of a chlorpyrifos dose which caused 100%

mortality in LSRI Report MAK/102/PYR (cited in the accepted chlorpyrifos rabbit teratology study under DPR Record No. 153166). **Acceptable** metabolite study. Aldous, 7/31/97.

Mouse Developmental Toxicity **

**342-254 036345 Deacon, M. M., J. S. Murray, M. K. Pilny, D. A. Dittenber, T. R. Hanley, Jr., and J. A. John, "The Effects of Orally Administered Chlorpyrifos on Embryonal and Fetal Development in Mice," Dow Chemical, Toxicology Research Lab., Midland, MI, 7/24/79; Chlorpyrifos, presumed technical; 0, 0.1, 1, 10, and 25 mg/kg/day by gavage; NOEL for maternal functional toxicity = 1 mg/kg/day [cholinesterase (ChE) effects as salivation, tremors, etc.]. ChE enzyme NOEL = 0.1 mg/kg/day (significant inhibition of maternal plasma ChE at 1 mg/kg/day). Developmental toxicity NOEL = 10 mg/kg/day (decreased fetal length and weight, delayed ossification in skull, sternebrae). ACCEPTABLE, in consideration of additional information in 291:050626 (See one-liner below). Report was previously not accepted (CDFA review 2/13/86, C. Aldous). C. Aldous, 6/1/87.

342-291 050626 (Addendum to 254:036345, primary mouse teratology study). Dow Chemical, Midland, MI, 7/24/79. New information provides grade of test article, dates of preparation of dose solutions, individual necropsy sheets for dams dying prior to term, and rationale for selection of mouse as test animal. C. Aldous, 6/1/87.

EPA 1-liner: Teratology - mice; Toxicology. Research Lab.; 7/24/74 [sic: presumed this is the 7/24/79 study]; Teratogenic NOEL > 25 mg/kg/day (HDT); fetotoxic NOEL = 10 mg/kg; fetotoxic LEL = 25 mg/kg (decreased fetal length, increased skeletal variants); Plasma and RBC ChE NOEL = 0.1 mg/kg/day.

342-013/053 031072 Summary of 254:036345 (see above).

342-682 152359 (Tab 43). Deacon, M. M., J. S. Murray, M. K. Pilny, K. S. Rao, D. A. Dittenber, T. R. Hanley, Jr., and J. A. John, "Embryotoxicity and Fetotoxicity of Orally Administered Chlorpyrifos in Mice", *Toxicol. Appl. Pharmacol.* 54:31-40 (1980). This is the published report corresponding to 342-254 036345, above.

Developmental Toxicity: Allegations of Effects on Humans

The following critical review by Dr. J. E. Gibson and associated support documents were submitted in response to allegations that chlorpyrifos elicited human malformations.

342-680 152356 Gibson, J. E., "Critical review of allegations associating Dursban with human teratogenicity", 12/23/96 (analysis was given DowElanco Study ID JEG122396). Dr. Gibson was responding to allegations by Dr. J. Sherman that chlorpyrifos was the causative agent for several human birth defects. The most detailed version of Dr. Sherman's report was in Int. J. Occup. Med. Toxicol., 4:417-431 (1995). Dr. Gibson's primary objections to the article were (1) Dr. Sherman does not have the training and experience to properly perform such an analysis, (2) the four cases described do not present a coherent pattern of effects, (3) the possibilities of genetic causation were ignored, even though in most cases one or more physicians experienced in evaluation of birth defects attributed findings to genetic defects (4) none of the cases offered measures of exposure, (5) statistical analysis in the article was unsound, (6) outcomes of cited

animal studies were misunderstood or misrepresented, and (7) the article did not state the author's role as paid consultant in lawsuits filed by the three affected families, which disclosure is an ethical responsibility of authorship. All lawsuits involving the four children have been dismissed. Neither the Sherman report (DPR Record No. 152349) nor Dr. Gibson's review are primary sources of new data, hence do not have independent worksheets. **Supporting data, including some complete studies, follow in Document Nos. 342-681 to 342-686. "One-liners" describing these submissions are found in this worksheet.** Aldous, 8/22/97.

Records submitted in support of 342-680 152356 above, included: Document No. 342-681: Record Nos. 152349, 152350, 152351, 152352, 152353 152354,152355; and Document No. 342-682: Record Nos. 152357, 152358, 152359.

NEUROTOXICITY

Acute neurotoxicity, rat **

342-448 126408 Wilmer, J., et. al. "Chlorpyrifos: Acute Neurotoxicity Study in Fischer 344 Rats", (Dow Chemical Company, Study ID: K-044793-093B, 9/11/92). Chlorpyrifos (purity 98.1%, lot #MM-890115-616) was administered in a single oral gavage to 10 Fischer 344 rats/sex/group at levels of 0, 10, 50 or 100 mg/kg. Body weights of mid- and high-dose rats were significantly reduced on day 2 but not on day 8 or 15. Clinical signs (increased perineal soiling) in mid- and high-dose rats and FOB observations (incoordination, decreased muscle tone, tremor, increased lacrimation and salivation) in high-dose females were seen soon after dosing (day 1). Motor activity was reduced in mid- and high dose rats on day 1; some reductions persisted to day 8 in high-dose females. NOEL (Body wt., Clinical signs, FOB and motor activity) = 10 mg/kg. No histopathologic changes. NOEL (histopathology) = 100 mg/kg. **No Adverse Effects. Original DPR review had requested additional purity, stability and homogeneity data on the dosing material, justification for dose level selection, and clarification of the statistical methods used, as criteria for "acceptable" status. These data were provided (see review for Record No. 132457, below) and report is now **acceptable**. Kellner and Gee, 7/5/94; Aldous, 4/9/97.

342-492 132457 [Cover letter referencing supplementary data was by Blewett, T. C. The acute range-finding study in this record supporting dose selection for the acute neurotoxicity study was by Wilmer, J. W. *et al.* (Study ID K-044793-093A)]. Addendum to Document # 342-448, Record # 126408 (rat acute neurotoxicity). Cover letter date: 10/4/94. The three primary acceptability concerns expressed in the original DPR review have been adequately addressed: characterization of technical and treated diets for content, stability, and homogeneity; range finding study clinical signs data as evidence that selected dose levels were appropriate; and evaluation of statistical significance for major parameters of this study. In the range-finding study, two F344 rats/sex/group were dosed once by corn oil gavage at 50, 100, 150, and 200 mg/kg. Clinical signs consistent with ChE inhibition peaked at about 6 hr after dosing. Major signs were decreased activity, incoordination, lacrimation, muscle twitches, perineal soiling, salivation, and tremors. These signs were well established at 100 mg/kg and above, especially in females. Range finding study data are sufficient to justify dose levels used in the neurotoxicity study. Additional statistical data are consistent with interpretations in the original DPR review.

The study is re-classified as **acceptable**, with **no adverse effects** other than expected ChE inhibition-associated changes. Aldous, 4/9/97.

90-day neurotoxicity, rat **

342-445 126304, “Chlorpyrifos: 13-Week Neurotoxicity Study in Fischer Rats”, (Shankar, M., Bond, D. and Crissman, J., Dow Chemical Company, Laboratory Project K-044793-094, 9/16/93). Chlorpyrifos, purity 98.1%, was administered in the feed at concentrations of 0, 0.1, 1, 5 or 15 mg/kg to 10 Fischer 344 rats/sex/group for 13 weeks. High-dose males and females had reduced motor activity at week 4. Perineal soiling (low incidence) was observed for 5 and 15 mg/kg/day groups; NOEL (for clinical signs, FOB, motor activity) = 1 mg/kg/day. No histopathologic findings. Neuropathological NOEL = 15 mg/kg/day. **No Adverse Effects. Report was originally classified as unacceptable, but upgradeable. Data provided in Record No. 132458 (see below) allowed an upgrade to **acceptable** status. This study type is considered “supplemental” under SB 950 at this time. Kishiyama, Kellner and Gee, 7/6/94; Aldous, 4/8/97.

342-493 132458 (Addendum to Document # 342-445, Record # 126304). Cover letter dated 10/4/94. The three primary acceptability concerns expressed in the original DPR review have been adequately addressed: characterization of technical and treated diets for content, stability, and homogeneity; ChE inhibition data as evidence that selected dose levels were appropriate; and evaluation of statistical significance for major parameters of this study. Data obtained from a 1988 subchronic feeding study found ChE enzyme inhibition NOEL = 0.1 mg/kg/day (inhibition of plasma ChE in both sexes and of RBC ChE in females at 1 mg/kg/day). ChE-related clinical effects NOEL = 1 mg/kg/day (perineal staining in occasional females at 5 and 15 mg/kg/day). Motor activity reduction, at 15 mg/kg/day during the week 4 evaluation only, was confirmed statistically. NOEL for findings other than probable acute ChE effects = 15 mg/kg/day (HDT). The study is re-classified as **acceptable**, with **no adverse effects** other than expected ChE inhibition and associated changes. Aldous, 4/8/97.

342-448 126409 Spencer, P. *et al.* “Positive Control Exercises: Motor Activity, Functional Observational Battery and Neuropathology”. Dow Chemical Co. submitted this report in support of -445:126304 and -448:126408; it contains validation studies of motor activity tests, functional observational battery (FOB) assays and neuropathological examinations using rats that were administered compounds with well-documented neurotoxic potential. This document was found to be ACCEPTABLE to satisfy the FIFRA guidelines for positive controls. An evaluation of these studies is included in the background sections of the acute and 13-week rat neurotoxicity studies mentioned above. No Worksheet. Kellner and Gee, 7/18/94.

4-week rat oral gavage cognitive study **

**342-747 162522 Maurissen, J. P., M. R. Shankar, and J. L. Mattsson, “Chlorpyrifos: cognitive study in adult Long-Evans rats”, The Dow Chemical Co., Midland, MI, 4/29/96, Laboratory Project ID: K-044793-096. Female Long-Evans rats were dosed by gavage in corn oil with 0, 1, 3, or 10 mg/kg/day chlorpyrifos (98.1% purity) for 4 weeks. The cognitive study was a “delayed matching to position task” design. Cognitive testing was done during each of the treatment weeks and for 4 weeks thereafter, by methods described below. Rats were placed on modest food restriction to provide incentive to seek the “food reward” in the study. Rats were trained and selected for the study, based on positional memory performance. In a given test, a rat

was presented with one of two retractable levers. The rat was to press the lever offered, cross the cage and interrupt a beam at the food cup within 10 seconds, and then return to the side of the cage with the levers. At this time, both levers would be presented. The rat was expected to select and press the correct lever (i.e., the one just presented a few seconds earlier) within 10 seconds after leaving the food cup station. A correct choice made a food reward available at the food cup. In addition to the above test, the task was made more difficult by involving progressively longer delays (up to 15 seconds) between the first lever press and the time in which a nose-poke in the food cup would extend the levers (called the delayed matching-to-position or “DMPT” paradigm). These rats were also examined twice daily on treatment days during the 4-wk dosing period: observations were about 3 hr and 21 hr after the most recent treatment. Satellite groups of 6/dose/interval were used for ChE assays and brain NTE assays on the day following the last treatment, and 1 month after the last treatment. The 1998 DPR review placed the NOEL for memory retention at 3 mg/kg/day (considering a small apparent memory retention change at 10 mg/kg/day to be a “possible adverse effect”). **This determination was subsequently changed** (see review for Document No. 342-789, immediately below). NOEL for clinical observations is 1 mg/kg/day (miosis). There is no NOEL for ChE inhibition (marked inhibition of plasma and RBC ChE and modest (8%) inhibition of brain ChE at 1 mg/kg/day). Some high dose observations associated with the DMPT tests were appropriately considered by investigators to have been attributable to motor slowing and/or decreased motivation (increased “actual total delay”, increased “void trials”, and decreased numbers of nose-pokes per trial). None of these were noted after the end of the treatment period. Report was originally classified as not acceptable (requiring dosing solution analysis). Such data were subsequently provided (see immediately below). Study is **acceptable**. Aldous, 11/6/98, 10/12/99.

342-789 168961, 168962, and 168963. **Supplemental information to the above cognitive study (Record 342-747 162522)**. Additional data and explanatory text were provided. Essential responses summarized below are detailed in review “W162522 s01.wpd”. New data supplied dosing solution analyses, and additional tables showing mean correct responses for individual animals and for treatment groups, including methodology used to obtain memory retention slope values. **These data allow an upgrade of Record No. 162522 to acceptable status**. In addition, investigators provided a statistical analysis of slopes of the memory retention curves for the various treatment groups. Data show that there were no statistically significant responses, hence data **do not demonstrate a possible adverse effect** (a change from the previous review). The variability of the data is sufficiently large that only a very substantial decrease of memory retention would have been detectable, thus the present study conditions **did not provide a sensitive test**. Aldous, 10/12/99.

Developmental neurotoxicity, rat **

**342-746 162521, Hoberman, A. M., “Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to CrI:CD®(SD)BR VAF/Plus® presumed pregnant rats”, Argus Research Laboratories, Inc., 5/1/98. Sponsor Protocol No. K-044793-109; Argus Study ID 304-001. CrI:CD®(SD)BR VAF/Plus® presumed pregnant rats were gavaged on gestation day 6 through lactation day 11 with chlorpyrifos (99.8%) in corn oil at 0, 0.3, 1, and 5 mg/kg/day. Initially there were 25 dams/group on treatment. On lactation day 5, twenty litters/treatment were continued on study. Four subsets of 20 pups/sex/group were selected on lactation day 5, each consisting of 1/sex/litter. Primary investigations for the subsets were: (Subset 1):

morphometric evaluations and histopathology of brains after postpartum day 12 sacrifice, (Subset 2): spatial delayed alternation studies at postpartum days 23-25 and 62-91, (Subset 3): motor activity testing on postpartum days 14, 18, 22, and 61: auditory startle on postpartum days 23 and 62, (Subset 4): evaluation of developmental landmarks (pinna unfolding, eye opening, preputial separation or vaginal opening); brain weight evaluation in 10/sex/group sacrificed during lactation days 66-71, and neurohistopathology following *in situ* perfusion of 6/sex/litter. Maternal NOEL = 0.3 mg/kg/day (brain ChE inhibition). Clinical signs of ChE inhibition were observed in 5 mg/kg/day dams. Developmental NOEL = 1 mg/kg/day (decreased neonatal survival; decreased pup growth, with 11% reduction in body weight at 66 days postpartum in males; maturational delays of pinna unfolding, preputial separation in males, and vaginal patency in females; reduced morphometric dimensions of cerebellum and hippocampal gyrus at day 12 postpartum compared to concurrent and historical controls, reduced morphometric dimensions of parietal cortex and hippocampal gyrus at day 66 postpartum compared to concurrent and historical controls in high dose females, reduced motor activity at day 14 postpartum, reduced auditory startle habituation peak response and increased latency to response at day 23 postpartum). This study was classified as “not acceptable but upgradeable” in the initial review, with the primary concern being appropriateness of the validation studies for evaluation of spatial delayed alternation. The response in Record No. 168955 (below) addressed the advantages of the using memory retention as a function of time for validation of technique, as compared with memory reduction due to exogenous chemicals. The investigators’ response gave examples of many confounding effects of exogenous chemicals on parameters other than on memory. Study findings are not of sufficient magnitude or persistence to be considered as “adverse”. Report is now **acceptable**. Aldous, 11/13/98 and 9/17/99.

342-769 164347 Submission of morphometry and histopathology data on F1 rats sacrificed after day 66 in Record No. 162521, above. Data were incorporated into the review for the main study under that Record Number. Aldous, 11/12/98.

342-789 168955, 168959, and 168960. Supplemental information to developmental neurotoxicity study 342-746 162521. Final report date of update: 5/7/99. Additional data and explanatory text were provided, **allowing an upgrade of Record No. 162521 to acceptable status**. Essential responses summarized below are detailed in review “s162521 s01.wpd”. The validation studies for evaluation of spatial delayed alternation, which were based on temporal patterns of memory performance over sufficient duration to show a consistent linear change over time, were shown to be satisfactory. Representative micrographs prepared by the pathologist were presented, demonstrating several of the commonly encountered lesions following insult to the several areas of the CNS, dorsal root ganglia, and peripheral nerves. Additional brain morphometric data requested by U.S. EPA were provided, plus selected published articles. One article showed that poor nutrition reduces pup brain weight increases, although to a much lesser extent than the decrement of body weight gain. Another article determined that the reductions of dimensions in brain regions appear to affect all brain morphometric measurements proportionately. A third article showed that poor nutrition leads to locomotion delays which are quite remarkable during lactation days 14-16, whereas some components of coordinated movement and altered posture remain affected for a longer time. Aldous, 9/17/99.

342-832 (suppl. to 342-746) 182481 (suppl. to 162521) Hoberman, A. M., Report Supplement 3 to: "Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD®(SD)BR VAF/Plus® presumed pregnant rats," Argus Research Laboratories, Inc., dated 5/1/98 (of original study), this supplement dated Oct. 9, 2000. Protocol No. of this supplement: 304-001. Brain morphometric data from the original report were re-tabulated alongside historical control data from 4 or 5 studies per parameter. Only one measurement having a high dose value statistically significantly different from concurrent controls was outside the range of the historical controls: the cerebellar anterior/posterior dimension in 5 mg/kg/day male 12-day pups was significantly below concurrent control dimension, and also outside the range of the available historical controls. Females did not suggest such a relationship at 12 days, and neither sex showed altered cerebellar anterior/posterior distance after 66 days. In the context of the demonstrated high maternal and neonatal toxicity of this dose, the supplemental data reinforce the lack of demonstrated special toxicity of the test article toward the developing nervous system. Supplemental to a previously acceptable study with no adverse effects. Aldous, 9/26/01.

342-824 178362 [Same report as 342-746 162521, above].

Delayed neurotoxicity, hen **

**342-291 051119 Barna-Lloyd, T., J. R. Szabo, and J. T. Young, "Chlorpyrifos: Subchronic Organophosphate-Induced Delayed-Neurotoxicity (OPIDN) Study In Laying Chicken Hens," (Report No. TXT:K-044793-064), Health & Environmental Sciences, Dow Chemical, Freeport, Texas, 4/86. Chlorpyrifos, tech. (approx. 96% purity). 0, 1, 5, and 10 mg/kg/day. No evidence of delayed distal neuropathy. 10 mg/kg/day chlorpyrifos caused weight loss, diminished egg laying capacity, and transient abnormal gait (fully reversible between dosing periods, and not persistent throughout study). Study fills neurotoxicity data requirement. C. Aldous, 6/3/87.

342-255 036346 Rowe, L. D., S. D. Warner, and R. V. Johnston, "Acute Delayed Neurotoxicologic Evaluation of Chlorpyrifos in White Leghorn Hens," Dow Chemical, Lake Jackson, Texas, 5/22/78; Chlorpyrifos, tech; 0, 50, and 100 mg/kg (gelatin capsule); NOEL = 100 mg/kg for behavioral or microscopically evident delayed neuropathy (Highest dose tested) NOT ACCEPTABLE, not complete, not upgradeable (no repeat dosage at day 21 when no effects were observed, not all currently required tissues examined.) C. Aldous, 2/13/86.

EPA 1-liner: [Acute delayed neurotoxicity - hen; Dow; 5/22/78] LD50 in hens= 50 mg/kg Negative @ 50 & 100 mg/kg. Core grade, minimum.

342-496 132855 Abou-Donia, M. B., and K. R. Wilmarth, "DowElanco chlorpyrifos joint neurotoxic action of chlorpyrifos and safrotin in hens (Duke Univ. Medical Center Dept. of Physiology and Pharmacology, Durham, NC). Assigned to Worker Health and Safety Branch for review. (Aldous, 8/8/97).

342-745 162520 (No Author) "Preliminary Report: Assessment of neurotoxicity associated with co-exposure to the organophosphorus insecticides chlorpyrifos and diazinon". White leghorn hens were dosed with maximal levels of chlorpyrifos and/or diazinon and kept alive with atropine and 2-PAM for 96 hours prior to sacrifice and assays of ChE (plasma and brain), and

brain NTE. There were apparently cumulative effects for brain and plasma ChE. Although diazinon by itself did not affect NTE activity, diazinon potentiated the NTE inhibition of chlorpyrifos from 35% to 65% of normal. There is insufficient information in this preliminary report to warrant a Medical Toxicology Branch worksheet. Aldous, 11/09/98.

IMMUNOTOXICITY **

** 342-0907; 258212; Chlorpyrifos: Assessment of Immunotoxic Potential Using the Sheep Red Blood Cell Assay after 28-Day Dietary Exposure to Rats@; (D.R. Boverhof, J.A. Murray, R. Sura; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID No. 101023; 6/28/10); Ten female Sprague-Dawley rats/group received 0, 0.4, 2.0 and 10.0 mg/kg/day of Chlorpyrifos technical (lot no. KC28161419; purity: 99.8%) in the diet for 28 days. Another 10 females were dosed by intraperitoneal injection with 20 mg/kg/day of cyclophosphamid from day 24 through day 28 as the positive control group. No deaths occurred during the treatment period. There was no treatment-related effect upon the mean body weights or food consumption. The hematology parameters were not affected by the treatment. Red blood cell cholinesterase (ChE) activity was reduced in a dose-related manner for all treatment groups. Brain ChE activity was significantly less than that of the controls at the 2 and 10 mg/kg treatment levels. The mean absolute and relative weights of the spleen and thymus were not affected by the treatment. The anti-SRBC IgM serum titers were less for the 2 and 10 mg/kg treatment groups. However, the effect was not manifested in a dose-related manner (i.e., the titers for 2 and 10 mg/kg groups were 36 and 59% of the control group, respectively). These results were judged to be equivocal based on the range of variability demonstrated in the control group values and the lack of a clear dose-response. Other parameters (spleen and thymus weights, white blood cell differential counts) did not indicate any suppression of immunopotency. The positive control was functional. **Study acceptable.** (Moore, 5/3/11)

ENDOCRINE DISRUPTOR STUDIES

SUPPLEMENTAL STUDIES

Human Epidemiological Studies Related to Neurotoxicity

342-543 138174 Nolan, R. J. (Study Director) “Critical analysis of the allegations of neuropathy due to chlorpyrifos submitted to the United States Environmental Protection Agency on November 7, 1994”. DowElanco had identified 31 individuals for whom physicians had made at least tentative diagnoses of neuropathy having possible association with chlorpyrifos. Although several cases of massive chlorpyrifos exposure had previously been documented, only one appeared to have caused organophosphate-type delayed neuropathy (OPIDN): this was an attempted suicide in which heroic treatments were required to address severe cholinergic symptoms (investigators citing Lotti *et al.*, 1986). The primary focus of the present investigation was on OPIDN symptoms, however other neurological findings were noted where found. None of the exposures (or worst plausible estimates of exposures) were judged to have been “biologically significant” [i.e., exposures were likely to have been too low to have measurably depressed plasma ChE, or (for inhalation route) were less than the NAS guideline of 10 µg/m³].

Studies to date have indicated that it is critical to achieve at least 50% inhibition of neurotoxic esterase in order obtain OPIDN symptoms: this is unlikely to happen except at dose sufficient to elicit major cholinergic crises. Onsets of acute symptoms in this study were compared with plausible response times for acute ChE inhibitory signs (usually within 4 hr, in any case within 24 hr). The majority of cases presented no cholinergic signs, and none presented signs which were unambiguously due to ChE inhibition. Only three persons had documented neuropathy which became evident within one month of alleged exposure (a plausible time frame for OPIDN), without a demonstrated alternate cause. Of these, no two of them had consistent symptoms. DowElanco therefore determined that the alleged neuropathologies could not reasonably be attributed to chlorpyrifos. No SB-950 worksheet is appropriate, since this is not a relevant study type, and data do not support a treatment effect. Aldous, 8/11/97.

342-707 154147 “Critical assessment of reported entitled ‘Review of chlorpyrifos poisoning data’”. This report was directed to Worker Health and Safety Branch for review, since the commonly expected poisoning incidents would be acute cholinergic events. No Medical Toxicology Branch review has been requested. Aldous, 8/11/97.

NON-GUIDELINE STUDIES RELATING TO CHOLINESTERASE AND METABOLISM

Human acute oral, evaluating clinical signs, metabolism, and/or cholinesterase

342-788; 168932; “A Rising Dose Toxicology Study to Determine the No-Observable-Effect-Levels (NOEL) for erythrocyte Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels”; (Kisicki, J.C. *et. al.*; MDS Harris, Lincoln, Nebraska; Study ID. DR K-044793-284; 4/19/99); Six male and six female human volunteers/treatment group were fasted overnight prior to being dosed orally once with 0 (placebo: lactose monohydrate), 0.5 or 1.0 mg/kg of chlorpyrifos powder (purity: 99.8%) in capsules (phase 1) or 0 or 2.0 mg/kg (phase 2) in a double blind, randomized study. The health status of each subject was monitored for up to 7 days. Vital signs (blood pressure, pulse rate, respiration rate, and body temperature) were recorded prior to dosing and at 1, 2, 4, 8, 12, 24, 48 and 168 hours after dosing. Blood samples for erythrocyte acetylcholinesterase (AChE) analysis were drawn 10 hours prior to dosing, at the time of dosing and at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144 and 168 hours post-dose for erythrocyte AChE activity and chlorpyrifos and metabolite analyses. A blood sample was drawn prior to dosing for paraoxonase activity determination. Urine samples were collected at 12 hour intervals starting 48 hours prior to dosing and at 0 to 6 and 6 to 12 hours post-dose and 12 hour intervals thereafter up to 168 hours after dosing. Although clinical symptoms such as anorexia, diarrhea, nausea, vomiting, dizziness, dyspnea, and headache were reported, none of these signs occurred in a dose-related manner. There was no apparent treatment-related effect upon any of the vital signs. Mean erythrocyte AChE activities were not significantly affected in a dose-related manner. One subject in the 2.0 mg/kg treatment group demonstrated a maximal 30% inhibition between AChE activity reported at 0 time and at 12 hours post-dose. Otherwise, no other subject in the high dose group had a

reduction in erythrocyte AChE activity greater than 12% based on the higher of the two baseline values. The blood and urine levels of chlorpyrifos and its metabolites and the paraoxonase activity analysis for individual subjects were not included in this initial report and thus could not be evaluated. **No adverse effects indicated. NOEL:** 1.0 mg/kg (based upon the 30% inhibition of erythrocyte AChE demonstrated by one of the subjects in the 2.0 mg/kg treatment group). **Supplemental Study.** (Moore, 5/18/99).

342-823 178361 This is a copy of study 342-788; 168932, above.

342-822 178360; Brzak, K. A., “A Rising Dose Toxicology Study to Determine the No-Observable-Effect- Levels (NOEL) for erythrocyte Acetylcholinesterase (AChE) Inhibition and Cholinergic Signs and Symptoms of Chlorpyrifos at Three Dose Levels – Part B” Acetylcholinesterase (AChE) Inhibition Study; Human; The Dow Chemical Company, Midland, MI; Laboratory I.D. No. 981176; 6/5/00; Chlorpyrifos; Human volunteers (6/sex/dose) received a single oral dose of 0.0, 0.5, 1.0 or 2.0 mg/kg (capsule form) in a double-blind clinical trial; blood and urine specimens were collected and analyzed for chlorpyrifos and its metabolites (chlorpyrifos oxon and 3,5,6-trichloro-2-pyridinol (TCP)) using GC-MS; pretreatment Chlorpyrifos Oxonase (CPOase), paraoxonase and diazoxonase were determined spectrophotometrically; blood and urine specimens were generally below the limit of quantitation (LOQ) for chlorpyrifos; average AUC for TCP in blood (by increasing dose) was 14.0, 25.2 and 51.2 µg/g, respectively and amount TCP excreted in the urine was 4.1, 8.7 and 15.9 mg, respectively during the first 168 hr following ingestion; blood and urinary TCP levels increased rapidly, remained constant over first 48 hr post-treatment, and then declined with an average half-life of 29 to 36 hr; administration by capsule probably reduced absorption (average of 34.7%, 30.8% and 29.5% absorbed in 0.5, 1.0 or 2.0 mg/kg dose group, respectively); serum CPOase activity was within the range of activity reported in previous studies and there were no extreme values; RBC ChE depression was seen in only one individual, a 2.0 mg/kg female that showed unusually high absorption of chlorpyrifos (87.9% versus 29.5%). Supplementary Data. Kellner, 2/23/01. [NOTE by C. Aldous: This study is “Part B” of 342-788; 168932, above].

342-834 183264 This is a copy of 342-822 178360, above.

Human repeat dosing, oral, evaluating clinical signs, metabolism, and/or cholinesterase

342-0343 071392 Coulston, F., T. Griffin, and L. Golberg, “Safety evaluation of Dowco 179 in human volunteers,” Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY, March 1972. Four male volunteers/group were dosed by tablet with Dowco 179 (chlorpyrifos) at 0 mg/kg/day (placebo) for 48 days, 0.014 mg/kg/day for 27 days, 0.03 mg/kg/day for 20 days, or 0.10 mg/kg/day for 9 days. Investigators assessed hematology and clinical chemistry weekly, and plasma cholinesterase (ChE) and RBC ChE twice weekly. These assessments continued as needed post-treatment to determine recovery. No treatments affected hematology or clinical chemistry or RBC ChE. Plasma ChE inhibition was marked and progressive over time at 0.10 mg/kg/day, with inhibition of 10% on days 1 to 3, 46% inhibition on day 6, and 66% inhibition on day 9, when dosing of that group was stopped. Recovery of this group progressed after cessation of dosing, with plasma ChE reaching twice the treatment day 9 activity at recovery day 11, and complete recovery to pre-treatment activity at recovery day 25.

Plasma ChE activity in the 0.03 mg/kg/day group was reduced by about 30% during days 16-20. Complete recovery from this lesser effect was complete by 20 days off treatment. Study gives useful supplementary information. Aldous, June 5, 2015.

342-0607 145821 is an exact copy of 342-0343 071392, above.

Human dermal (or dermal/oral comparison), evaluating clinical signs, metabolism, and/or cholinesterase

342-122 948115 Nolan, R. J., D. L. Rick, N. L. Freshour, and J. H. Saunders, "Chlorpyrifos: pharmacokinetics in human volunteers following single oral and dermal doses," Dow Chemical, Midland, MI, Aug. 1982. Healthy male volunteers were dosed with chlorpyrifos (analytical grade, 99.8% purity) to assess kinetics of chlorpyrifos and of its major metabolite (3,5,6-trichloro-2-pyridinol), and to follow changes in plasma and RBC cholinesterase (ChE) over time. N = 5 for major parameters. Exposures were a 0.5 mg/kg single oral dose, followed 4 weeks later (ample time for clearance from the oral exposure) by a single 5 mg/kg dermal dose. None of these doses elicited clinical signs. Following 0.5 mg/kg oral dosing, plasma ChE was inhibited to about 15% of baseline, with greatest inhibition at 0.5 to 2 hrs after dosing. By 8 hours, plasma ChE levels were 3-4 fold higher than the lowest activity. By 27 to 30 hours, plasma ChE activity had returned to baseline. Dermal dosing with 5 mg/kg chlorpyrifos had no definitive effect on plasma ChE at any time post-dose. RBC ChE activity was inherently more variable than plasma ChE. RBC ChE activity was not measurably affected by these oral or dermal exposure levels. Blood chlorpyrifos levels following 0.5 mg/kg oral dosing was either non-detectable, or was in the range of 5-30 ng/ml blood. The highest blood chlorpyrifos levels did not appear at consistent times post-dosing, and clearly would not represent a reliable measure of exposure. Blood concentrations of chlorpyrifos following 5 mg/kg dermal exposure were either non-detectable or did not exceed 10 ng/ml. Blood levels of 3,5,6-trichloro-2-pyridinol following 0.5 mg/kg oral dosing showed quite variable kinetics between subjects, but tended to peak at 2-8 hours at about 1 µg/ml blood, with levels at 24 hours being no less than 50% of peak concentrations. This confirms that this metabolite would be a good indicator of exposure. Dermal exposure of 5 mg/kg yielded 3,5,6-trichloro-2-pyridinol blood levels which occasionally exceeded 0.1 µg/ml. There was about a 4-fold range of peak 3,5,6-trichloro-2-pyridinol blood between dermal exposure subjects. Investigators estimated the half-life of 3,5,6-trichloro-2-pyridinol to be about 27 hours by either route. Urinary peak excretion rates of 3,5,6-trichloro-2-pyridinol were at about 9 hours for oral route, and about 42 hours for the dermal route. Time to decrease to about 50% of maximum urinary 3,5,6-trichloro-2-pyridinol levels were roughly 30 hours for oral exposure and 84 hours for dermal route. Thus this study shows that chlorpyrifos is only moderately absorbed through the skin, that plasma ChE is a good marker of systemic load for several hours after exposure, whereas urinary 3,5,6-trichloro-2-pyridinol assays would be useful for qualitative exposure assessment for 2-3 days for oral route, and slightly longer for dermal exposure. Useful supplementary data. Aldous, 4/16/15.

342-0197 001367, also 342-0627 149353 These are exact copies of 342-122 948115, above.

342-0343 071383 Nolan, R. J., D. L. Rick, N. L. Freshour, and J. H. Saunders, "Chlorpyrifos: pharmacokinetics in human volunteers," *Toxicology and Applied Pharmacology* **73**, 8-15 (1984). This is a published version of Record No. 948115.

342-763 165484 Griffin, P., H. Mason, K. Heywood, and J. Crocker, "Oral and dermal absorption of chlorpyrifos: A human volunteer study", cover letter dated 11/23/98. (This was a manuscript accepted for publication in Occupational & Environmental Medicine). Data were reviewed by T. Thongsinthusak of DPR Worker Health and Safety Branch: that review is bound with the volume. Dermal applications led to 1% absorption (evidenced as dialkylphosphate urinary metabolites), and 53% unaltered chlorpyrifos was recovered by washing the application site. Investigators did not account for the balance for the remainder of residues. Aldous, 10/13/99.

Primate Studies

342-384 091999 Coulston, F., L. Golberg, R. Abraham, K. F. Benitz, T. B. Griffin, and M. Norvell, "Final Report on Safety Evaluation and Metabolic Studies on DOWCO 179 (IN 151)," Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY, 3/18/71 (unpublished study). This early study included rat and monkey data. Only the 6-month monkey study is summarized here. Fourteen rhesus macaque monkeys (*Macaca mulatta*) were placed on study (8 males and 6 females), with 3-4 animals per group at doses of 0, 0.08, 0.40, or 2.00 mg/kg/day of DOWCO 179 (chlorpyrifos, purity unspecified). Test article was administered by gavage as aqueous suspensions in 1% gum tragacanth. Four males (1/group) were sacrificed at 3 months. Nine of the remaining 10 monkeys survived to 6 month termination. There were no effects on body weight, clinical signs, hematology, or clinical chemistry. Plasma cholinesterase (ChE) inhibition was observed at all dose levels (65%, 28%, and 24% of pre-treatment activities for 0.08, 0.40, or 2.00 mg/kg/day monkeys (sexes combined), respectively. RBC ChE was only inhibited at the top 2 dose levels (79% and 34% of pre-treatment activities for 0.40, or 2.00 mg/kg/day monkeys, respectively. Midbrain ChE (the only CNS tissue evaluated) showed no evidence of treatment effect at 0.4 mg/kg/day or below. Only 2 monkeys were evaluated for midbrain ChE at 2.00 mg/kg/day: a male sacrificed at 3 months which showed no difference from the control, and a female sacrificed at 6 months which had a lower activity than concurrent controls, but within the range of variability indicated for other animals on study. If the one case indicating a treatment effect were indeed dose-related, it would indicate comparable response to whole-brain values previously obtained for repeat-dose studies in species such as rat and dog. Urine was collected for 24 hours during week 16 to see whether 3,5,6-trichloro-2-pyridinol (TCP) in urine could be used to estimate chlorpyrifos exposure. Results were highly variable for the 7 subjects evaluated, but show promise for urinary TCP as a rough estimator of exposure. Investigators evaluated possible induction of biphenyl-4-hydroxylase or biphenyl-2-hydroxylase activity in liver homogenate 9000 x g fractions, and found no induction of these activities. This is a supplementary study, performed before modern guidelines were formulated, and is not a candidate to fill a FIFRA data requirement. Data are too scant to assess possible adverse effects. Aldous, 3/19/18.

Rat acute oral, evaluating clinical signs, metabolism, and/or cholinesterase

342-763 164102 Mendrala, A. L. and K. A. Brzak, "Chlorpyrifos: Part A - Concentration - time course of chlorpyrifos and chlorpyrifos-oxon in blood," The Dow Chemical Co., Midland, 8/31/98, Laboratory Project Study ID 971187A. This study had two segments. [Segment 1]: Chlorpyrifos was administered by gavage in corn oil to male F344 rats at dose levels of 0.5 to

100 mg/kg. Four rats/group were killed at intervals ranging from 10 min to 12 hr to determine time course of (a) concentrations of chlorpyrifos and chlorpyrifos-oxon, and (b) plasma and brain cholinesterase (ChE) activities. Chlorpyrifos concentrations peaked at 3 hr, with levels dropping substantially at 6 to 12 hr. Chlorpyrifos-oxon was only about 1% as abundant as chlorpyrifos, and was typically detectable at 1 hr and 3 hr intervals only. Plasma ChE inhibition was evident at all dose levels, with plasma ChE inhibition peaking in the range of 3 to 6 hours. The 3-hour plasma response (as % of control ChE activity) was 84%, 72%, 42%, 33%, 18%, and 18 % in 0.5, 1, 5, 10, 50, and 100 mg/kg groups, respectively. Brain ChE inhibition was evident at 10 mg/kg and above, with brain ChE activity (as % of control) at 6-hour peak response being 88%, 30%, and 28% in 10, 50, and 100 mg/kg groups, respectively. Estimated half-life for chlorpyrifos in blood was 2.7, 1.5, 2.1, or 7.3 hours for 5, 10, 50, or 100 mg/kg chlorpyrifos dose levels, respectively. [Segment 2]: Four rats/group were dosed by gavage in corn oil with achieved levels of 3 and 63 mg/kg of ring-labeled ¹⁴C-chlorpyrifos, administered 3 hours prior to sacrifice. Blood was collected for measurements of circulating chlorpyrifos, chlorpyrifos-oxon, and the trichloropyridinol (TCP) hydrolysis product. TCP was by far the most abundant residue in blood (about 99% of chlorpyrifos-equivalents at either dose level). Remaining dose-equivalents were approximately 1% chlorpyrifos, and less than 0.1% was chlorpyrifos oxon. Report provides useful supplementary data. Findings of brain ChE are designated as “possible adverse effects.” Aldous, 10/13/99; re-examined with a worksheet by Aldous on April 9, 2018.

Rat chlorpyrifos acute vapor inhalation, evaluating clinical signs, metabolism, and/or cholinesterase

NOTE: The two rat acute vapor inhalation studies below assess acute responses to **parent chlorpyrifos** and to **chlorpyrifos oxon**, respectively.

342-0937; 271252; Hotchkiss, J. A., S. M. Krieger, K. M. Mahoney, K. A. Brzak, N. A. Malowinski, and D. L. Rick, “Nose-Only Inhalation of Chlorpyrifos Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain and Lung Cholinesterase Activity in Female CD(SD): Crl Rats”; (Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID No. 131040; 5/2/13); Forty female Crl:CD(SD) rats/group were exposed nose-only to either 0 (filtered air) or 17.7 ppb (0.254 µg/l) of a saturated vapor of chlorpyrifos technical (lot no. 7299412; purity: 97.6%) for 6 hours. Eight animals/group/time point were euthanized at 0, 2, 4, 6 and 12 hours post-exposure. Blood, brain and lung tissue were procured from each animal. Cholinesterase activity was assayed in the plasma, blood, brain and lungs. Blood levels of chlorpyrifos and its primary metabolite, trichloropyridinol were determined as well. The animals demonstrated no signs of toxicity during the exposure or for the 12-hour post-exposure period. The peak level of chlorpyrifos in the blood was immediately after the completion of the exposure, diminishing to a non-detectable level by 6 hours post-exposure. The trichloropyridinol peak levels were noted up to 2 hours post-exposure and gradually diminished over the 12-hour post-exposure observation period. Chlorpyrifos-oxon was not detectable in any of the samples. None of the tissues which were assayed from the exposed group demonstrated a significant reduction in cholinesterase activity in comparison to the control activity levels. Activity in the blood and plasma of the exposed animals was 93 and 86%, respectively, of the control values at 4 hours post-exposure, the maximal reduction. The ChE activity in the lungs of the exposed animals was 89% of the

control group at that time point. There was no apparent effect upon ChE activity in the brain. **No adverse effect indicated. Study supplemental.** (Moore, 6/4/13)

342-0950 274123; “Nose-Only Inhalation of Chlorpyrifos-Oxon Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain and Lung Cholinesterase Activity in Female CD(SD):Crl Rats”; (J.A. Hotchkiss, S.M. Krieger, K.M. Mahoney, K.A. Brzak, N.A. Malowinski, D.L. Rick; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID. 131067; 8/30/13); In Phase 1, the highest attainable saturated vapor concentration of chlorpyrifos-oxon (oxon) under standard laboratory conditions typical of an acute nose-only inhalation exposure study was determined and selected for Phase 2 of this study. In Phase 2, eight female CD(SD):Crl rats/group/sacrifice time were exposed for 6 consecutive hours to filtered air (control) or a time weighted average concentration of $35.3 \mu\text{g}/\text{m}^3$ (2.58 ppb) oxon vapors using a flow-past nose-only inhalation exposure system. Rats were sacrificed immediately (0 hr) and at 1, 2, 4, 8 and 24 hours after the end of exposure. Blood and tissues were isolated and processed to determine cholinesterase (ChE) activity in red blood cells (RBC), plasma, and lung and brain tissues. Whole blood samples from n=4 rats in each group/sacrifice time were analyzed to determine the concentrations of oxon and 3,5,6-trichloro-2-pyridinol (TCP). No clinical signs of toxicity were noted in oxon-exposed rats at any time during or after exposure. No oxon was detected in the blood at any time after exposure (lower limit of quantification (LLQ), 0.118 ng/g blood), however, blood TCP levels > LLQ (2.44 ng/g blood) were detected in all assayed blood samples collected at 0 through 4 hours after exposure and in 1/4 assayed blood specimens collected 8 hours post-exposure. By contrast, blood TCP levels were below LLQ in 3/4 and 4/4 animals sacrificed at 8 and 24 hours after exposure, respectively. No oxon-induced inhibition of ChE activity was detected in RBC, plasma, lung or brain at any time after exposure. The presence of TCP in the blood of oxon-exposed rats confirms that oxon vapor is absorbed through the respiratory tract, however, the inhaled oxon is rapidly metabolized and not systemically bioavailable, given that all the assayed blood levels were below LLQ (0.118 ng/g or $3.53 \times 10^{-4} \text{ nmol/g}$ blood). Based on the absence of cholinesterase inhibition in RBC, plasma, brain or lung (the portal-of-entry tissue), the 6-hour No Observed Effect Concentration (NOEC) for inhaled oxon vapor is > $35 \mu\text{g}/\text{m}^3$ air. The results of this study suggest that there is no biologically relevant hazard from inhalation of a saturated vapor concentration ($35.3 \mu\text{g}/\text{m}^3$) of chlorpyrifos oxon. **Study Supplemental.** (Guo, 11/13/13)

Rat chlorpyrifos repeat-dose vapor inhalation, evaluating clinical signs, metabolism, and/or cholinesterase

342-0343 071388 Landry, T. D., D. A. Dittenber, L. L. Calhoun, L. G. Lomax, and P. Morabito, “Chlorpyrifos: 2-week nose-only vapor inhalation exposure study in Fischer 344 rats,” The Dow Chemical Company, Midland, MI, 6/10/86. This study exposed female rats (N = 6) to 0 or 12 ppb chlorpyrifos vapor (99.7% purity) 6 hours/day, 5 days/week, with sacrifice one day after the last exposure (with 3 consecutive days of exposure before the day of sacrifice). Investigators evaluated cholinesterase (plasma, RBC, and brain), clinical signs, body weights, hematology, and gross pathology. There were no treatment responses. The tested concentration was noted to be about 50% of the maximum theoretical maximum vapor level for chlorpyrifos. Although individual data were provided, there is no DPR worksheet for this report, since it does not address a data requirement, and because it was negative. Aldous, 5/15/15.

342-0343 071389 Corley, R. A., T. D. Landry, L. L. Calhoun, D. A. Dittenber, and L. G. Lomax, "Chlorpyrifos: 13-week nose-only vapor inhalation exposure study in Fischer 344 rats," The Dow Chemical Company, Midland, MI, 11/13/86. This study exposed both sexes (N = 10) to 0, 5.2, 10.3, or 20.6 ppb chlorpyrifos vapor (100% purity, reporting mean assayed chamber concentrations) 6 hours/day, 5 days/week, with sacrifice one day after the last exposure (with at least 4 consecutive days of exposure before the day of sacrifice, following overnight fasting). Investigators evaluated cholinesterase (plasma, RBC, and brain), clinical signs (shortly after each exposure period), body weights, organ weights, hematology, clinical chemistry, urinalysis, and gross pathology. Protocol tissues of both sexes were subject to histopathology examination in control and high dose groups. There were no treatment responses. The maximum vapor level for chlorpyrifos was noted to be about 25 ppb. This is a valid supplementary study. Although individual data were provided, there is no DPR worksheet for this report, since it does not address a standard data requirement, and because responses were negative. Aldous, 5/15/15.

Rat chlorpyrifos acute aerosol inhalation, evaluating clinical signs, metabolism, and/or cholinesterase

342-0908; 258214; AAcute Inhalation Exposure of Adult Crl:CD(SD) Rats to Particulate Chlorpyrifos Aerosols: Kinetics of Concentration-Dependent Cholinesterase (ChE) Inhibition in Red Blood Cells, Plasma, Brain and Lung@; (J.A. Hotchkiss, S.M. Krieger, K.A. Brzak, D.L. Rick; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID. 091133; 6/29/10); In Phase I, six Sprague-Dawley rats/sex/group were exposed nose-only to 0, 13.3 or 66.7 mg/m³ (analytical) of Chlorpyrifos technical (lot no. KC28161419; purity: 99.8%) for six hours. Blood was drawn from an indwelling jugular catheter at 2, 4, 6 hours of exposure and at 0.5, 1, 2, 4, 6, 12, and 24 hours post-exposure. Red blood cell and plasma cholinesterase (ChE) activities were assayed for each time point. In Phase II, 54 female rats/group were exposed nose-only to 0, 3.7, 12.9, 22.1 or 53.5 mg/m³ of the test material for up to 6 hours. Six animals/group/time point were euthanized at 2, 4, and 6 hours of exposure and at 2, 6, 12, 24, 48 and 72 hours post-exposure. Cholinesterase activities in the red blood cells, plasma, lungs and brain were assayed and the blood concentrations of chlorpyrifos (CPF), chlorpyrifos-oxon (CPO) and trichloropyridinol (TCP) were measured. Urine was collected from 6 animals/group at 0 to 12, 12 to 24, 24 to 48 and 48 to 72 hours and trichloropyridinol concentrations were determined. In Phase I, significant inhibition of red blood cell and plasma ChE activities was evident at 13.3 mg/m³. For RCE ChE activity, maximal inhibition of 65% for males and 80% for the females was noted at 2 hours post-exposure. For plasma ChE activity, maximal inhibition of 66% for males and 87% for females was evident from 6 hours of exposure to 1 hour post-exposure. Based on these results, females were deemed to be more sensitive to the effects of CPF on ChE activity and thus were selected for testing in Phase II. ChE inhibition in the plasma achieved a maximal level of 48% at 6 hours of exposure in the 3.7 mg/m³ group. In the lungs, a maximal level of ChE inhibition was noted at 47% in the 3.7 mg/m³ at 6 hours of exposure. ChE activity in the brain was significantly reduced for the 12.9, 22.1 and 53.5 mg/m³ groups with maximal inhibitions of 19, 21 and 22%, respectively, which were noted at 6, 6 and 2 hours post-exposure, respectively. For RBC ChE activity, the results were inconsistent at the 3.7 mg/m³ exposure level possibly due to the variability of the control values. Maximal reduction in activity was not evident until 24 to 48 hours post-

exposure. The blood levels of CPF were highest at 4 to 6 hours of exposure for all of the exposure levels with a peak value of 65 ng/g noted for the 53.5 mg/m³ group. CPO was recovered in the blood at peak levels of 0.22 ng/g during the exposure at the 53.5 mg/m³ exposure level. Peak levels of 2400 ng/g of TCP for the highest exposure group were noted at 12 hours post-exposure. The plasma half-life of CPF ranged from 0.463 to 3.34 hours over the exposure concentration range. The ratio of the areas under the curve for TCP/CPF ranged from 545 to 1057. The inhaled dose of the test material was calculated to be 1.04, 3.62, 6.21 and 15.0 mg/kg. Excretion of TCP in the urine demonstrated a half-life ranging from 10.6 to 11.6 hours. Using these excretion data the percentage of inhaled CPF which was absorbed was calculated and ranged from 36 to 79%. **Study supplemental.** (Moore, 5/2/11)

Rat chlorpyrifos life stage comparisons (as neonate vs. young adult), evaluating clinical signs, metabolism, and/or cholinesterase

342-0906; 257044; A Comparison of Cholinesterase (ChE) Inhibition in Young Adult and Pre-weanling CD Rats after Acute and Repeated Chlorpyrifos or Chlorpyrifos-Oxon Exposures@; (M.S. Marty, A.K. Andrus; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Study ID. 091107; 6/29/10); Pre-weanling (11 days post-natal) and young adult female Sprague-Dawley rats were dosed orally by gavage, using vehicles of corn oil or rat=s milk or in the diet (adult rats only) with concentrations of Chlorpyrifos technical (CPF) (lot no. KC28161419, purity 99.8%) ranging from 0.05 to 10 mg/kg, in a single dose regimen or at concentrations ranging from 0.05 to 3.5 mg/kg/day of CPF in corn oil in a 10-day multiple dosing regimen (pre-weanling: days 11 to 21 post-natal, young adult: 70 to 80 days old). Other groups of pre-weanling and young adult female rats were dosed orally by gavage in a single dose regimen with Chlorpyrifos-oxon (CPO) in corn oil (lot no. 199902031-66, purity: 94.9%) at concentrations ranging from 0.005 to 1.0 mg/kg. In a 10-day multiple dosing regimen, both pre-weanling and young adult females were dosed orally by gavage with 0.01 and 0.5 mg/kg/day of CPO in the same manner as the CPF-treated animals. Eight animals/sex were included in the pre-weanling groups and 8 females/group were dosed in the young adult cohort. Preliminary studies were performed in order to establish the time-to-peak inhibition profile for plasma, red blood cell and brain cholinesterase (ChE) inhibition. In the dose-response studies, animals were euthanized at the time-to-peak ChE inhibition. The concentrations of CPF, CPO and trichloropyridinol (TCP) in the blood of some of the study animals were determined. A functional observational battery was performed on the study animals in the multiple-dosing regimen after 9 days of dosing. The times-to-peak effect were as follows: PND 11 pups: 1. CPF in corn oil (6 hours), 2. CPO in corn oil (4 hours), 3. CPF in rat=s milk (8 hours); young adult females: 1. CPF in corn oil (8 hours), 2. CPO in corn oil (4 hours), 3. CPF in diet (after conclusion of the 12-hour exposure period) (8 hours). Based upon the results of the dose response studies, no effect levels were established for plasma, red blood cell and brain ChE inhibition under the different dosing scenarios. In the **single dose regimen**, NOELs for the plasma and red blood cell ChE inhibition were 0.5 mg/kg for both sexes of the pre-weanlings after treatment with CPF, using either corn oil or rat=s milk as the vehicle, and for the young adult females treated by gavage, using a corn oil vehicle, or in the diet. The NOEL values for the brain ChE inhibition were 2 mg/kg for the male pre-weanlings treated with CPF, using either corn oil or rat=s milk as the vehicle, for the female pre-weanlings, using corn oil as the vehicle and for the adult females treated by gavage or in the diet. For the pre-weanling females dosed with CPF in rat=s milk, the brain ChE inhibition NOEL was 0.5 mg/kg. The NOELs for

treatment with a single dose regimen of CPO were as follows: for both male and female pre-weanlings, the NOELs for plasma ChE inhibition: 0.05 mg/kg, for red blood cell ChE inhibition: 0.1 mg/kg and for brain ChE inhibition: 0.5 mg/kg. For the young adult females, the NOEL for plasma, red blood cell and brain ChE inhibition were 0.1, 0.1 and 0.5 mg/kg, respectively. In the **multiple dose regimen** in which the pre-weanlings and young adults were treated with CPF in corn oil by gavage, the NOEL values for ChE inhibition were as follows: male and female pre-weanlings, plasma and RBC: 0.1 mg/kg, brain: 0.5 mg/kg; young adult females, plasma: 0.1 mg/kg/day, red blood cell: 0.5 mg/kg/day, brain: 0.5 mg/kg/day. The NOELs for ChE inhibition after multiple treatments with CPO in corn oil were as follows: male and female pre-weanlings and young adult females, plasma and red blood cell: 0.01 mg/kg/day, brain: 0.5 mg/kg/day. The NOEL values were reduced from 0.5 mg/kg to 0.1 mg/kg/day for plasma and red blood cell ChE inhibition in the pre-weanlings after multiple treatments with CPF in corn oil. The brain ChE inhibition for these animals was lowered from 2 mg/kg to 0.5 mg/kg/day. In the young adult females, the NOELs for plasma and brain ChE inhibition were lowered from 0.5 mg/kg to 0.1 mg/kg/day and from 2 mg/kg to 0.5 mg/kg/day, respectively. The concentrations of CPF and TCP in the blood at the NOEL and/or LOEL treatment levels for the various treatment scenarios were examined. Treatment with CPF in corn oil or rat's milk to pre-weanling rats in either a single dose or multiple dose regimen resulted in TCP/CPF concentration ratios (based on ng/g of blood) ranging from 70 to 209. For the young female rats, in certain instances, the CPF concentration was below the limits of detection and the ratio could not be calculated. Otherwise, the ratios were 935 and 449 (0.5 and 2.0 mg/kg, by gavage, respectively), 7243 (2.0 mg/kg in the diet) in the single dose regimen and 2450 (0.5 mg/kg/day) and 651 (1.0 mg/kg/day) after multiple doses by gavage. These data indicate a possible difference in the metabolic disposition of CPF between the pre-weanling pups and the young adult animals. No treatment-related effects were identified in the FOB. **Supplemental Study.** (Moore, 2/23/11)

342-0897 253051 This is an interim report of 342-0906; 257044, above.

342-0997 293277 M.S. Marty, A.K. Andrus, M.P. Bell, J.K. Passage, A. W, Perala, K. A. Brzak, M. J. Bartels, and D.R. Juberg, "Cholinesterase inhibition and toxicokinetics in immature and adult rats after acute or repeated exposures to chlorpyrifos or chlorpyrifos-oxon," Regulatory Toxicology and Pharmacology | Vol 63, 209-224 (2012). This is a published version of Record No. 257044, above.

342-764 164103 Mattsson, J. L., J. P. Maurissen, P. J. Spencer, K. A. Brzak, and C. L. Zablony, "Effects of chlorpyrifos administered via gavage to CD rats during gestation and lactation on plasma, erythrocyte, heart and brain cholinesterase, and analytical determination of chlorpyrifos and metabolites", The Dow Chemical Co., Midland, 08/98. This study was not reviewed under SB-950, but has been examined extensively by R. Cochran for the chlorpyrifos risk assessment. Aldous 10/13/99.

Dog chlorpyrifos subchronic or subacute, dietary, evaluating clinical signs, metabolism, and/or cholinesterase †

342-836; 183362; "Chlorpyrifos Technical: 6-Week Dietary Study of Acetylcholinesterase Inhibition in Beagle Dogs"; (B.R. Marable, P.C. Baker, K.E. Stebbins and J.P. Maurissen; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland,

MI; Study ID: 011036; 7/27/01); Four beagle dogs/sex/group received 0, 0.5, 1.0 or 2.0 mg/kg/day of Dursban FM (Chlorpyrifos Technical) (lot no. 7299412, TSN100759, purity: 97.6%) in the diet for 6 weeks. The animals were fed twice per day and the content of the a.i. in the diet was adjusted in a manner such that the daily intake per body weight was maintained. No deaths resulted from the treatment. There was no apparent dose-related effect upon the mean body weights. No clinical signs were noted during the treatment period. The mean red blood cell cholinesterase (ChE) activity was reduced in a dose-related manner with maximal levels of inhibition achieved after 6 weeks (% of baseline, males, 0.5: 44.5%, 1.0: 27.6%, 2.0: 14.4%; females, 0.5: 56.9%, 1.0: 32.8%, 2.0: 18.9%). There was no dose-related effect upon the brain, diaphragm, muscle or nodose ganglion acetylcholinesterase (AChE) activity for either sex after 6 weeks of treatment. The AChE activity in the left atrium of the heart of the males was reduced in a dose-related manner (% of control, 0.5: 99.3, 1.0: 84.5%, 2.0: 74.5%). This effect was not noted for the females. **Possible adverse effect:** significant inhibition of AChE in the heart. **NOEL:** (M/F) < 0.5 mg/kg/day (based upon the reduced red blood cell ChE activity for both the males and females in the 0.5 mg/kg treatment group); **Supplemental Study** (non-guideline study) (Moore, 11/4/02)

342-833 182482 Baker, P. C. *et al.*, “Communication: Preliminary evaluation of acetylcholinesterase (AChE) in brain, peripheral tissues, and RBC in beagle dogs,” The Dow Chemical Company, Midland, MI, 5/11/01. Report ID CPF0501. [Report begins on p. 38 of this volume]. Three males/group were dosed in diet with 0, 0.3, 0.6, or 1.2 mg/kg/day chlorpyrifos for 28 days. Parameters evaluated at termination focused on acetylcholinesterase measurements in RBC’s, brain, nodose ganglion, left atrium, left ventricle, diaphragm muscle, and thigh muscle. In-life RBC acetylcholinesterase activity was measured weekly. All dogs survived the treatment, and there were no characteristic clinical signs. Body weight was unaffected by treatment. RBC acetylcholinesterase activity was reduced in dose-related fashion. Despite high variability in control activities, reductions in the higher two dose levels were clearly treatment-related (about 50% reduction at 1.2 mg/kg/day). These changes appeared to be progressive over time. No other tissues showed statistically significant reductions in AChE activity. Some of the assayed AChE activity values were so variable that the small numbers of dogs available could only have indicated major treatment responses. This is a useful pilot study, but data are unsuitable for quantitative analysis. Aldous, 9/27/01.

Dog chlorpyrifos subchronic or subacute, pet collar exposure, evaluating clinical signs, metabolism, and/or cholinesterase

342-244; 34080; Boyd, J. P., Cholinesterase Inhibition Study; 855; Dog; P.A.C.E. International, Dallas, TX; Project No. 20-208-1184; 5/14/85; pet collar, 8.0% A.I.; 6 treated animals, 4 untreated control animals; 1 collar/animal, 91 day treatment period; No mortality; Observations: no treatment-related effects, no irritation evident at the collar site; Cholinesterase (ChE) Inhibition: significant inhibition of plasma ChE from day 3 to end of study (maximal inhibition-83.7%, day 69), no apparent treatment effect on RBC ChE activity; no adverse effect; NOEL cannot be determined (significant inhibition of plasma ChE activity exhibited by treated animals); Study supplemental. (Moore, 5/12/93)

In vitro tissue studies of cholinesterase inhibition and metabolism

342-0951 274124; “*In vitro* Sensitivity of Cholinesterase to Inhibition by Chlorpyrifos-oxon in Several Tissues of the Rat”; (J.E. Chambers, E.C. Meek, H.W. Chambers; Center for

Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS; Study ID. NS000128; 9/16/13); to compare the inherent sensitivity of cholinesterase in several tissues to inhibition by chlorpyrifos-oxon (CPFO) through determination of inhibitory concentrations (IC_{50} values), young adult male rats were euthanized; brain, blood, lung, heart, diaphragm, esophagus, stomach (flushed) and duodenum were removed from the animals and flash frozen in liquid nitrogen. In some animals, the heart and lungs were perfused with saline through the aorta to remove residual blood and the contents of the esophagus and duodenum were flushed out of the tissues, followed by flash freeze. Red blood cells (RBCs) collected were used intact, and also lysed and centrifuged to prepare a RBC ghost. All tissues were homogenized (except plasma and RBC ghosts) in 0.05 M Tris-HCl buffer, pH 7.4 at 37 °C, with a motorized glass-Teflon homogenizer, and plasma was diluted and RBCs and RBC ghosts were re-suspended in this buffer. A modified Ellman (spectrophotometric) method for measurement of cholinesterase activity was used with acetylthiocholine or butyrylthiocholine (only for some of the plasma duodenum samples) as substrate and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) as the chromogen. Tissue preparations were diluted in the above buffer to yield an activity level that produced about 1.2-2.0 Absorbance Units (AU) following the substrate incubation period (15 min. at 37 °C for all tissues except RBCs which was 1 hr at 37 °C) in the control samples. Five concentrations of CPFO in ethanol were used to provide an inhibition range of 20-80%; protein was quantified by the Lowry method. IC_{50} values were calculated for each of 3 replications (3 separate rats) by log-legit regression, and 95% confidence intervals were calculated for the IC_{50} means. The mean IC_{50} values (for assays conducted with acetylthiocholine as substrate, AChE) were: brain, 3.77 nM; duodenum – flushed, 3.72 nM vs. not flushed, 4.17 nM; esophagus – flushed, 3.13 nM vs. not flushed, 3.28 nM; stomach-flushed, 4.08 nM; lung – perfused, 7.21 nM vs. not perfused, 8.57 nM; heart – perfused, 3.06 nM vs. not perfused, 3.91 nM; diaphragm, 6.64 nM; RBCs, 4.19 nM vs. RBC ghosts, 5.08 nM; plasma, 55.36 nM. The assays conducted with butyrylthiocholine showed IC_{50} values very similar to those by AChE: duodenum – flushed, 3.72 nM vs. not flushed, 5.05 nM; plasma, 50.05 nM. There is no difference in the inherent sensitivity of the acetylcholinesterase in the several solid tissues studied (brain, esophagus, stomach, duodenum, heart, diaphragm, lung and red blood cells) to inhibition by chlorpyrifos-oxon, as indicated by IC_{50} values all within the same order of magnitude. The higher IC_{50} values in plasma logically result from the presence within plasma of other proteins that can be readily inhibited by CPFO (e.g., carboxylesterases) or that can absorb CPFO (e.g., albumin), thus reducing the levels of CPFO that were available to inhibit plasma cholinesterase; lower CPFO bioavailability resulted in a higher IC_{50} value, but it does not necessarily indicate lower inherent sensitivity of plasma cholinesterase. **Study Supplemental.** (Guo, 1/02/14)

342-774 165918 “Standard operating protocol for analysis of the effects of chlorpyrifos, diazinon, and sulfotep on neurite length in differentiating neuroblastoma cells in vitro.” This volume is currently in evaluation by another division of DPR, and appears unlikely to be pivotal to Medical Toxicology Branch, based on its title. There are, however, studies in the public literature relating to chlorpyrifos effects on differentiating cells in culture, hence this protocol may be supportive of such a study. C. Aldous, 10/13/99.

Registrant rebuttal responses or commentaries on cholinesterase effects and inter-species extrapolations

342-790 168952 Chen, W. L., R. J. Nolan, and J. L. Mattsson, "Dow AgroSciences' response to the report of the Hazard Identification Assessment Review Committee (HIARC) entitled 'Chlorpyrifos - Hazard Identification Based on Animal Studies'". This record was an evaluation of existing data, and not a report of new data, except for an abstract of a recent human study by Kisicki *et al.* (reviewed as DPR Record No. 168932, see 1-liner below). "Laboratory Study ID" # GH-C 4904. This record was provided to call to question key U.S. EPA conclusions regarding hazard evaluation of chlorpyrifos. **Human clinical sign evaluation:** The cited abstract concluded that the NOEL for RBC AChE was 1 mg/kg, based on 1/12 volunteers having over a 17% decrease in this enzyme at 2 mg/kg. None of the 12 volunteers at the highest dose of 2 mg/kg experienced clinical symptoms. This result suggest that a single subject presenting signs of "blurred vision, feeling of faintness, and runny nose" in an earlier study at 0.1 mg/kg/day was unlikely to have been responding to chlorpyrifos treatment. **Relevance of RBC AChE vs. BuChE:** Registrants observed that the latter has no known physiological function and no apparent relevance to human hazard assessment. In contrast, RBC AChE is evidently identical to the AChE associated with neuromuscular transmission, hence relevant in human hazard assessment. **Comparative inhibition of AChE from different sources:** Rat studies over the dose range of 10 to 100 mg/kg indicated that RBC AChE had a 12-fold lower ED₅₀ than whole brain, hence regulation on blood AChE would protect against cholinergic toxicity. AChE in other tissues was less sensitive to inhibition (i.e. had a higher ED₅₀) than whole brain (p. 22). **Primary conclusions of investigators:** Investigators determined (1) that human data are valid and preferable to animal data in assessing human hazard, (2) that human RBC AChE rather than BuChE should be used to set RfD's, (3) and that the laboratory animal data base (if agencies are determined to use such for human safety assessment) is sufficiently complete that (a) there is no justification for an additional ten-fold safety factor for uncertainties regarding possible special toxicity to infants and children and (b) the comparative blood ChE responses of humans and laboratory animals (for RBC AChE and BuChE) are sufficiently well-characterized that a 10-fold interspecies uncertainty factor is not appropriate. Supportive published articles were included: (1) Chen *et al.* "Human red blood cell acetylcholinesterase inhibition as the appropriate and conservative surrogate endpoint for establishing chlorpyrifos reference dose", **Regulatory Toxicology and Pharmacology** **29**, 15-22 (1999), (2) Schardein and Scialli, "The legislation of toxicologic safety factors: The Food Quality Protection Act with chlorpyrifos as a test case", **Reproductive Toxicology** **13**, 1-14, 1999, and (3) Gibson, J. E. *et al.*, "How to determine if an additional 10x safety factor is needed for chemicals: A test case with chlorpyrifos", **Toxicological Sciences** **48**, 117-122 (1999). No worksheet (no reviewable data). Aldous, 9/14/99.

342-756 162540 Albers, J. W. *et al.*, "Determination of the reference dose for chlorpyrifos: Expert panel report." No date was given for report: cover letter date for volume was 6/19/98. Dow AgroSciences convened a panel of experts, who determined in this 85-page record that (1) multiple studies support an RfD for repeated oral dose exposure of 0.01 mg/kg/day, and (2) the RfD for single oral exposure was determined to be 0.05 mg/kg. There are no new studies, hence no DPR worksheet. Aldous, 10/13/99.

Mechanistic Studies on Serine Hydrolases that Degrade Endocannabinoids

The following studies by R. L. Carr *et al.* explored effects of chlorpyrifos on two serine hydrolase enzymes involved in degradation of endocannabinoid degradation: [monoacylglycerol lipase (MAGL), and fatty acid amide hydrolase (FAAH)]. The associated endocannabinoids were 2-arachidonoylglycerol (2-AG) and anandamide (AEA). The latter are essential in neurodevelopment, but their levels in CNS are controlled by the above enzymes to keep ligand concentrations at optimal levels. Test animals were male and female Sprague-Dawley rat pups, dosed with chlorpyrifos daily by gavage from PND 10 through 16 at up to 5 mg/kg/day. Tissues tested included forebrain, and sometimes midbrain and plasma. Generally cholinesterase (ChE) was assayed in parallel.

(No DPR Record or Document Number) Carr, R. L., A. L. Adams, D. R. Kepler, A. B. Ward, and M. K. Ross, "Induction of endocannabinoid levels in juvenile rat brain following developmental chlorpyrifos exposure," *Toxicological Sciences* **135**(1), 193-201, 2013. Ten-day old Sprague-Dawley rat pups were dosed with chlorpyrifos (99% purity) daily by gavage in corn oil from PND 10 through 16 at 0, 1, 2.5, or 5 mg/kg/day, with groups of 6-8 (blocked by sex and litter) sacrificed at 4, 12, 24, or 48 hours after the last dose. Forebrain ChE, MAGL, and FAAH activities were assayed at these intervals, in addition to forebrain levels of the two endocannabinoids which are primarily degraded respectively by MAGL and FAAH: (2-AG and AEA). Forebrain ChE response was strongest at 12 hours after the last dose, with inhibition of 24%, 55%, and 68% at respective dose levels. ChE inhibition at 48 hours was 9%, 36%, and 46% respectively. MAGL response was strongest at 4 hours, with inhibition of 14%, 24%, and 41% at respective dose levels. MAGL inhibition at 48 hours was 7%, 16%, and 33% respectively. FAAH was more strongly inhibited: inhibition was greatest at 4 to 12 hours after the last dose. Inhibition at 12 hours was 52%, 90%, and 93% at respective dose levels. FAAH inhibition at 48 hours was 16%, 38%, and 48% respectively. Levels of 2-AG were most notably increased at 12 hours, at which time respective treated groups had elevations of 30%, 52%, and 63% over controls (all statistically significant). By 48 hours, there were no significant differences from control, however the 5 mg/kg/day group mean was 19% over control. Levels of AEA were also most notably increased at 12 hours, at which time respective treated groups had elevations of 65%, 128%, and 190% over controls (all statistically significant). By 48 hours, the only significant difference from control was at 5 mg/kg/day group (81% over control). Investigators indicated in their discussion that FAAH is the dominant degradation enzyme for AEA, evidenced by other studies showing nearly complete mitigation of AEA effects when a specific FAAH inhibitor is employed. Investigators noted further that other studies had found that 2-AG is subject to appreciable degradation by enzymes not included in the present study. Investigators concluded that particularly alteration of FAAH activity due to chlorpyrifos may alter neuronal system development at critical stages of growth. There is no DPR worksheet, as only summary data were provided. This is a valid supplementary study. Aldous, 5/13/15.

(No DPR Record or Document Number) Carr, R. L., C. A. Graves, L. C. Mangum, C. A. Nail, and M. K. Ross, "Low level chlorpyrifos exposure increases anandamide accumulation in juvenile rat brain in the absence of brain cholinesterase inhibition," *Neurotoxicology* **43**:82-89 (2014). This work is basically an extension of that described in *Toxicological Sciences* **135**(1), above, assessing the lower dose of 0.5 mg/kg/day from PND 10-16, with sacrifice at 4 and 12 hours. Serum carboxylesterase was inhibited by 94% and 74% at 4 and 12 hours after the last

dose, respectively. Serum cholinesterase was inhibited by 36% and 25% at 4 and 12 hours after the last dose, respectively. Forebrain cholinesterase and forebrain MAGL activities were not altered at this dose. Forebrain FAAH was reduced by 14% at 4 hours (not significant) and by 25% at 12 hours (significant, $p < 0.05$). There was no significant difference in 2-AG in forebrain at 0.5 mg/kg/day, but forebrain AEA levels were increased by 18% at 4 hours and by 37% (significant, $p < 0.05$) at 12 hours. There is no DPR worksheet, as only summary data were provided. This is a valid supplementary study. Aldous, 5/13/15.

(No Document or Record Numbers) Carr, R. L., A. Borazjani, and M. K. Ross, "Effect of developmental chlorpyrifos exposure, on endocannabinoid metabolizing enzymes, in the brain of juvenile rats," *Toxicological Sciences* 122(1): 112-120 (2011). Male and female Sprague-Dawley rats were exposed to 0, 1, 2.5, or 5 mg/kg/day chlorpyrifos. Most tests were performed in pups dosed on PND 10-16, with sacrifice 4 hours after the PND 16 treatment. Body weight gains were reduced (dose-related) in 2.5 to 5 mg/kg/day pups. ChE activity (as percent of control) was reduced in respective dose groups of pups by tissue as follows: forebrain (18, 41, and 52%), medulla-pons (18, 38, and 55%), and serum (32, 50, and 55%). Pup forebrain MAGL activity was reduced by 14, 22, and 37% in respective groups. Pup forebrain FAAH activity was reduced by 40, 93, and 96% in respective groups. Investigators used a fluororhosphonate-biotin (FP-biotin) probe to mark serine hydrolase enzymes in PND 16 pups and performed an SDS-PAGE separation, ultimately visualizing the marked enzymes with a chemiluminescent reagent and capturing images on x-ray film. FP-biotin probe analyses found a strong reduction of marked FAAH at 1 mg/kg/day, with no visible presence remaining at higher dose levels. MAGL staining was quite faint, even in controls, but suggested a treatment-related reduction in female pups. Another serine hydrolase enzyme, KIAA 1363, described elsewhere as highly responsive to chlorpyrifos oxon, showed a marked dose-related reduction in this treatment range. Possible importance of the latter was outside of the scope of this article, however other abstracts by Cassidy et al. indicate that spontaneous recovery of KIAA 1363 may be rapid enough to not warrant major concern. MAGL was detectible in membrane fractions but not in cytosolic fractions, when evaluated in pup brain extracts. A specific MAGL inhibitor, JZL184, reduced 2-AG hydrolysis activity to about 55% of control activity at 10 μ M, with no additional inhibition at higher dose levels. This suggests that chlorpyrifos effects on MAGL are less likely to elicit profound effects on its substrate levels than effects on FAAH. Investigators concluded that chlorpyrifos inhibition of AEA hydrolysis may be the principal concern for juvenile development, with reduced FAAH enzyme activity as the most plausible cause. There is no DPR worksheet, as data are limited to summary tables and figures. Aldous, 5/14/15.

ADDITIONAL NON-GUIDELINE REPORTS: NOT REVIEWED FOR THIS SUMMARY

342-0976 286275 Miguel A. Sogorb and Eugenio Vilanova, "Serum albumins and detoxication of anti-cholinesterase agents," *Chemico-Biological Interactions* 187, Issues 1–3, 6 September 2010, Pages 325-329. This published article is of possible general interest in understanding the role that serum albumin plays in hydrolyzing certain cholinergic compounds. The summary data are too brief to review. The abstract follows (Aldous, 4/10/18). Serum albumin displays an esterase activity that is capable of hydrolysing the anti-cholinesterase compounds carbaryl, paraoxon, chlorpyrifos-oxon, diazoxon and O-hexyl, O-2,5-dichlorophenyl phosphoramidate. The

detoxication of all these anti-cholinesterase compounds takes place at significant rates with substrate concentrations in the same order of magnitude as expected during in vivo exposures, even when these substrate concentrations are between 15 and 1300 times lower than the recorded Km constants. Our data suggest that the efficacy of this detoxication system is based on the high concentration of albumin in plasma (and in the rest of the body), and not on the catalytic efficacy itself, which is low for albumin. We conclude the need for a structure–activity relationship study into the albumin-associated esterase activities because this protein is universally present in vertebrates and could compensate for reduced levels of other esterases, i.e., lipoprotein paraoxonase, in some species. It is also remarkable that the biotransformation of xenobiotics can be reliably studied in vitro, although conditions as similar as possible to in vivo situations are necessary.

Record Number 275321 Epidemiology studies pertaining to chlorpyrifos exposures: considerations of reliability and utility
DPR Received Date: 12/13/2013
Study Date:
Document Number: 342-0952

Record Number 279907 Development of chemical specific adjustment factors for chlorpyrifos and chlorpyrifos oxon
DPR Received Date: 09/04/2014
Source: The Dow Chemical Company Midland, Michigan
Study Date: 10/31/2013
Document Number: 342-0960

Record Number 282730 In vitro age-dependent enzymatic metabolism of chlorpyrifos and chlorpyrifos-oxon in human hepatic microsomes and chlorpyrifos-oxon in plasma (journal article)
DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 281309 Chlorpyrifos reevaluation in California toxicology research in support of chlorpyrifos (pt.1-2)
DPR Received Date: 11/18/2014
Source: Dow AgroSciences Indianapolis, IN
Study Date: 11/17/2014
Document Number: 342-0964

Record Number 282735 In vitro rat hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon (journal article)
DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 282734 Age-dependent pharmacokinetic and pharmacodynamic response in preweanling rats following oral exposure to the organophosphorus insecticide chlorpyrifos (journal article)

DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 282731 The effects of plasma lipids on the pharmacokinetics of chlorpyrifos and the impact on interpretation of blood biomonitoring data (journal article)

DPR Received Date: 01/20/2015
Study Date: 02/17/2009
Document Number: 342-0965

Record Number 282729 A human life-stage physiologically based pharmacokinetic and pharmacodynamic modeling for chlorpyrifos: development and validation (journal article)

DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 282486 Using PBPK/PD modeling for assessing the toxicity of chlorpyrifos and the risks from current and historical exposures

DPR Received Date: 01/20/2015
Study Date: 12/08/2014
Document Number: 342-0965

Record Number 282559 Chlorpyrifos PBPK/PD modeling for multiple routes of exposure

DPR Received Date: 01/20/2015
Source: Summit Toxicology, L.L.P. Allenspark, CO
Study Date: 11/08/2013
Document Number: 342-0965

Record Number 282740 Serum albumin is as efficient as paraoxonase in the detoxication of paraoxon at toxicologically relevant concentrations (journal article)

DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 282741 Cytochrome P450-specific human PBPK/PD models for the organophosphorus pesticides: chlorpyrifos and parathion (journal article)

DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 282653 Application of a source-to-outcome model for the assessment of health impacts from dietary exposures to insecticide residues (journal article)

DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 282557 Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling of dermal exposure to chlorpyrifos: validation and application to mixed oral and dermal exposures

DPR Received Date: 01/20/2015
Source: Battelle Pacific Northwest Laboratories Richland, WA

Study Date: 03/05/2013
Document Number: 342-0965

Record Number 279905 A human life-stage physiologically based pharmacokinetic and pharmacodynamic model for chlorpyrifos: development and validation (journal article)
DPR Received Date: 09/04/2014
Document Number: 342-0960

Record Number 282736 A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans (journal article)
DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 282558 Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling of oral exposure to chlorpyrifos: impact on toxicity adjustment factors
DPR Received Date: 01/20/2015
Source: Battelle Pacific Northwest Laboratories Richland, WA
Study Date: 01/25/2013
Document Number: 342-0965

Record Number 282737 Physiologically based pharmacokinetic and pharmacodynamic model for the inhibition of acetylcholinesterase by diisopropylfluorophosphate (journal article)
DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 282728 Chlorpyrifos PBPK/PD model for multiple routes of exposure (journal article)
DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 282727 Development of a source-to-outcome model for dietary exposures to insecticide residues: an example using chlorpyrifos (journal article)
DPR Received Date: 01/20/2015
Document Number: 342-0965

Record Number 274124 In vitro sensitivity of cholinesterase to inhibition by chlorpyrifos-oxon in several tissues of the rat
DPR Received Date: 10/03/2013
Document Number: 342-0951

Record Number 279906 Chlorpyrifos PBPK/PD model for multiple routes of exposure (journal article)
DPR Received Date: 09/04/2014
Document Number: 342-0960

Record Number 282738 Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 282739 Human paraoxonase 1 hydrolysis of nanomolar chlorpyrifos-oxon concentrations is unaffected by phenotype or q192r genotype (journal article)

DPR Received Date: 01/20/2015

Document Number: 342-0965

Record Number 948107) Clinical toxicity of Dursban in dog after multiple applications of aerosol formulation (18P.)

DPR Received Date:

Source: Dow Chemical U.S.A. Midland, MI

Study Date: 12/01/1968

Document Number: 342-0119

Record Number 948135) Comparison of cholinesterase depression in humans and rabbits following exposure to Chlorpyrifos (22 pp.)

DPR Received Date:

Source: Dow Chemical U.S.A. Midland, MI

Study Date: 08/01/1971

Document Number: 342-0032