

**CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH**

**SUMMARY OF TOXICOLOGY DATA
AZINPHOS-METHYL**

Chemical Code # **000314**, Document Processing Number (DPN) # **154**
SB 950 # **174**

September 1, 1987

Revised 1/27/88, 6/27/88, 11/1/88, 2/23/90, 1/08/91, 4/25/91, 9/4/91, 11/1/94, 6/29/95, 1/9/98,
11/15/01, April 7, 2004 and October 20, 2004

I. DATA GAP STATUS

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

Toxicology one-liners are attached.

All record numbers for the above study types through 210505 (Document No. 154-0322) were examined. This includes all relevant studies indexed by DPR as of October 20, 2004.

In the 1-liners below:

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

Previous revisions by Rinkus, Silva, Aldous, Kellner, and Gee.
Present revision by Gee under file name: T041020.wpd

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

See also “Guidance for the Re-registration of Pesticide Products Containing Azinphos-methyl” (Environmental Protection Agency, Office of Pesticide Programs, September, 1986). The Re-registration standard indicates that studies were required in all areas except chronic dog, oncogenicity in the mouse and unscheduled DNA synthesis.

An IRED was signed in October of 2001.

COMBINED, RAT

**** 154-214 074676 “Study of Chronic Toxicity and Carcinogenicity to Wistar Rats,” Bayer AG; Study no. T 2015169, 12/10/84). Azinphos-Methyl (purity 87.2%) was administered in the feed at concentrations of 0 (vehicle = 1% DAB8 peanut oil), 5, 15 or 45 ppm to 10 (12 months) and 50 (24 months) SPF Wistar (Bor: WISW [SPF Cpb]) rats/sex/group. No adverse effect. NOEL = 15 ppm (Body weight was consistently lower after test substance was administered in the feed of high dose males). ChE NOEL = 5 ppm (significant inhibition of erythrocyte ChE in both sexes at ≥ 15 ppm; plasma ChE in males at 45 ppm and in females at ≥ 15 ppm; brain ChE in both sexes at 45 ppm, in females only at 12 months). ACCEPTABLE. (Kishiyama & Silva, 2/23/90).**

NOTE: An NCI bioassay was published in 1978. High dose levels in that study were about 3-fold higher than the high dose groups in the above Bayer study. The one-liner for the NCI study is found under “Oncogenicity, Rat” (below). That study found possible increases in tumor incidences in high dose males (pancreatic islet cell adenoma or adenocarcinoma; thyroid follicular cell adenoma, cystadenoma or adenocarcinoma; and adrenal cortical adenoma and adenocarcinoma). There are several reasons why results of the NCI study cannot be considered to have been superseded by record 074676, above. The difference in dosage ranges was already mentioned. Both of the studies indicated appreciable evidence of toxicity in one or both sexes (clear body weight decrements in the NCI study in both sexes: modest, but apparently treatment-associated decrement in body weights of high dose males in the above Bayer study). The Bayer study was toward the low end of an acceptable range of dosages, whereas the NCI study probably exceeded an appropriate “MTD” during the first 20 weeks of treatment in males (before high dose ration of azinphos-methyl was reduced from 250 to 125 ppm). Also, the two studies differed in strain of rats employed, type of diet, duration of dosing, and type of caging. The design of the NCI study left more open to speculation about possible tumorigenicity than did the Bayer study, due to the small size of the concurrent control group and to the lack of a third treated group. In summary, the data from the accepted Bayer study above shows

no evidence of neoplasia at dosages up to 45 ppm, whereas the older NCI study presents equivocal evidence of treatment effects in males at the highest dosage (time-weighted average dose of 156 ppm), which apparently exceeded the MTD during part of the study. The possibility that tumors observed in high dose males in the NCI study may have been treatment-related cannot be dismissed on the basis of the Bayer study, however the latter study appears to be much more relevant for human risk assessment of possible chronic low-dose exposure. Aldous, 1/8/91.

CHRONIC TOXICITY, RAT

154-097 916526, "Toxicity of Gusathion During Repeated Administration to Rats for Two Years," (Huntingdon Research Centre, England, 6/2/66). Azinphos-methyl (no purity stated) fed at 0, 5, 20 or 50 ppm (increased to 100 ppm at 47 weeks) in the diet of 40 Wistar rats/sex/group for 97 weeks; a low dose of 2.5 ppm was started 6 months into the study with its own control of 30/sex; ChE NOEL = 5 ppm; Insufficient information to assess adverse effects; UNACCEPTABLE (no diet analysis, pathology summary tables inadequate, limited clinical chemistry (cholinesterase only), no purity of test article, high mortality, no clear evidence of an MTD with marginal (20 - 30%) depression in cholinesterase reported, no effect on body weight.) (J. Christopher, 5/6/85).

CHRONIC TOXICITY, DOG

154-045 916525, "Chronic Oral Toxicity Study in Dogs," (Huntingdon Research Centre, England, 7/13/66). Azinphos-methyl (no purity stated), 4 dogs/sex/group (cocker spaniels), high dose level raised from 50 ppm to 300 ppm in 4 steps, intermediate dose level raised from 20 to 50 ppm in 2 steps, low dose level - 5 ppm; insufficient information for adverse effects assessment; incomplete (missing some individual data, no age at start, no pathology summary table, no diet analysis and no description of test material), UNACCEPTABLE - not upgradeable (doses too low and changed too often). (J. Christopher, 5/2/85).

EPA considers the NOEL = 5 ppm and the study adequate (see 1986 EPA Re-registration Standard document).

** 154-254 089216 Allen, T. *et al.* "52-week Oral Toxicity (feeding) Study with Azinphos-methyl (E 1582) in the Dog" (RCC, Research and Consulting Company AG, Lab project ID # 100644, 5/31/90). Azinphos-methyl, E 1582, Batch # 233 896 032, 91.1% pure at 0, 5, 25 and 125 ppm in feed to 4 beagle dogs/sex/group. Liver effects consisting of decreased serum albumin, increased cytochrome P-450 and decreased liver weight were observed, but there were no corresponding histopathological findings. Brain AChE was inhibited 26% in males and 20% in females at 52 weeks in the 125 ppm dose group; cholinergic NOEL: 5 ppm. No Adverse effect. Originally unacceptable because no justification was given for the dose levels chosen; animals may not have been adequately challenged. Upgraded to ACCEPTABLE status upon review of ChE inhibition and clinical sign data in study 257:98124 submitted by Mobay Corp. on 8/12/91. (Kellner and Gee,

9/4/91).

154-257 098124 [Addendum to 254:89216]. This study supplies the range-finding data that were listed as deficient in the original review of 254:089216. Azinphos-methyl was administered to beagle dogs (1 male and female per dose) at levels of 0, 20, 50, 100, 200 and 400 ppm for 19 weeks. Whole-blood cholinesterase was inhibited 50% compared to control by week 19 in the 100 ppm dose group; clinical signs at 100 ppm consisted of occasional muscle spasms and tremors. These additional data justify the doses used in the 52-week study and permit an upgrade of study 254:089216 to acceptable status. Supplemental Data. Kellner and Gee, 9/3/91.

ONCOGENICITY, RAT

154-108/145 916531, 038252 “Bioassay of Azinphos-methyl for Possible Carcinogenicity,” (NCI, Gulf South Research Institute, 1978). Azinphos-methyl (90%) at 78 or 156 ppm (time-weighted averages) to 50 males/group and at 62.5 or 125 ppm to 50 females/group for 80 weeks; 10 rats/sex used as controls; evidence of pancreatic islet cell adenoma or adenocarcinoma, thyroid follicular cell adenoma, cystadenoma or adenocarcinoma, adrenal cortical adenoma and adenocarcinoma in males only at high dose; UNACCEPTABLE (no individual data including pathology, use of pooled control data, only 2 dose levels, exposure for only 80 weeks). (J. Christopher, 5/31/85). [According to current guidelines, the length of exposure would be considered inadequate. Gee, 1/9/98]

EPA did not accept this study based on some of the same reasons but “...concluded that there is no clear link between the development of tumors and the administration of azinphos-methyl.” (see 1986 EPA Re-registration Standard document).

ONCOGENICITY, MOUSE

154-108/145 038721, 038253 “Bioassay of Azinphos-methyl for possible Carcinogenicity,” (NCI, Gulf South Research Institute, 1978). Azinphos-methyl (90%) at 31.3 or 62.5 ppm to 50 male mice/group and at 62.5 or 125 ppm to 50 female mice/group, 10 mice/sex used as a control group; UNACCEPTABLE (missing clinical observations, use of pooled control data and no individual data); - Not upgradeable (only two dose levels). (J. Christopher, 3/5/85).

EPA found this part of the study acceptable with no oncogenic effect.

** 154-146 027120, “Oncogenicity Study of Azinphos-Methyl (Guthion) in Mice,” (Study No. 80-271-02, Mobay Chem. Corp., 4/10/85). Azinphos-methyl (86.74%) at 0 (corn oil), 5, 20 and 40 (80 for week 1) ppm to 50 mice/sex/group (actual concentrations of 4.14, 17.3 and 34.2 ppm) for 104 weeks; CD-1 mice; No adverse effects indicated; oncogenicity NOEL \geq 40 ppm; complete, ACCEPTABLE. (A. Apostolou, 9/12/85).

EPA review was not completed at the time of the writing of the re-registration standard.

REPRODUCTION, RAT

** 154-179 061965, 061966, "Two-Generation Study on Rats", (Bayer AG, 2/84; report issued 3/10/87, Report No. 94814). Azinphos-methyl, 87.2%, Batch No. 79/R225/42, was fed in the diet at 0, 5, 15 or 45 ppm to 12 males and 24 females per group, 2 generations, 2 litters per generation. Analyses of diets indicated an adequate content. Reproductive NOEL = 5 ppm (decreased viability and lactation indices); parental NOEL = 5 ppm - see below (decreased weight gain, increased mortality at 45 ppm in females, cholinergic signs in some animals at 45 ppm). Initially reviewed as having a possible adverse effect on reproduction (Gee, 1/26/88) based on the marginal findings in the parental animals. A 28-day subchronic study was submitted (CDFA # 066534 - see below) in which cholinesterases were demonstrated to be inhibited at 20 ppm. Reconsideration of the total data and the discussion in CDFA # 062083 by Van Goethem, the parental NOEL is lowered from 15 ppm to 5 ppm, the same as the Reproductive NOEL. ACCEPTABLE. (Gee, 1/26/88 and 6/27/88).

154-173 062108 Apparently an exact duplicate of 179:061966 (same date, same number of pages, nearly the same title in library printout).

154-195 066534, "R 1582 (Common Name: Azinphos-methyl, the Active Ingredient of Guthion) Toxicity Study on Rats with Particular Attention to Cholinesterase Activity (28-Day Feeding Study as a Range-Finding Test for a 2-Year Study)" (Bayer AG, FRG, 5/18/83, Report 95608). Azinphos-methyl, 93.3% was fed in the diet at 0, 2, 20 or 50 ppm to BOR:WISW (SPF/Cpb) rats, 5/sex/group for 28 days. Plasma and erythrocyte cholinesterase levels were measured on days 1, 4, 14 and 28 and brain cholinesterase at termination. No effects on appearance or behavior were reported; food consumption and body weights were comparable; plasma and RBC cholinesterase were considered to be significantly inhibited at 20 and 50 ppm in females and males with the effect noted earlier in females; inadequate description of assay for independent evaluation. Submitted to support reproduction study Records 061965, 061966. Supplementary data. (Gee, 6/24/88).

154-256 089539 Holzum, B. "E 1582 (R 1582) (C. N. Azinphos-methyl) Investigation of Inhibition of Cholinesterase Activity in Plasma, Erythrocytes and Brain in a 1-generation Study". (Mobay No. 100646, Bayer AG, Dept. of Toxicology, Germany, 10/8/90.) Azinphos-methyl, 91.7% purity (3/24/88 analysis), was fed in the diet at 0, 5, 15 and 45 ppm to SPF-bred Wistar rats (strain Bor:WISW; SPF Cpb) to 18 male and 46 female animals per group, 1 generation, 1 litter (plus 3 additional groups of 10 males treated with 5, 15 and 45 ppm and 20 untreated females/group). Statistically significant ChE inhibition was seen in all 15 and 45 ppm parental rats; for 5 ppm dosed F0 rats, only erythrocyte ChE for females on day 28 p.p. showed notable inhibition: Cholinergic NOEL = 5 ppm. Viability index and body weight gain of pups were significantly reduced at 15 and 45 ppm doses: Reproductive NOEL = 5 ppm. Inhibition of pup brain AChE detected in 45 ppm group on day 5 and 28 p.p. This study supports the conclusions in record 061965, 061966 that reduction in certain reproductive parameters occurs at the same level as significant ChE inhibition in F0 parental rats, but it does not establish a definitive link between adverse

reproductive effects and maternal toxicity/stress at 15 ppm azinphos-methyl. Supplemental Study. (Kellner and Gee, 8/22/91).

REPRODUCTION, MOUSE

154-045 916533, "Effect of Guthion in the Diet on the Reproduction and Lactation of Mice," (Tab. #16963, University of Chicago, 9/15/65). Azinphos-methyl (80%) at 0, 5, 10, 25 or 50 ppm to 6 male mice and 24 female mice/group; insufficient information for adverse effects assessment; incomplete (missing diet analysis, individual data), UNACCEPTABLE - not upgradeable (too few males per group, fed diet only 30 days before mating, females had previously mated). (J. Christopher, 5/2/85).

EPA considers the study to be "invalid."

TERATOLOGY, RAT

** 154-189 065021, "A Teratology Study with Azinphos-methyl (Guthion Technical) in the Rat", (Miles Inc., IN, 12/22/87, Report 94987). Azinphos-methyl, 87.7%, Batch 79-R-225-42/5FEB87; given by oral gavage at 0 (6% Emulphor), 0.5, 1.0 or 2.0 mg/kg, days 6 - 15, to Charles River Crl:CD BR rats; 33/group with 5 sacrificed on day 16 of gestation and 28 on day 20. Plasma, erythrocyte and brain cholinesterase were measured on days 16 and 20 for inhibition with significant inhibition at 2.0 mg/kg/day. Dose selection was based on a range-finding study. No evidence for developmental toxicity at any dose. Maternal ChE NOEL = 1.0 mg/kg; developmental NOEL \geq 2.0 mg/kg (in range-finding study, no external findings were reported up to 4.5 mg/kg). ACCEPTABLE. (Gee, 6/24/88).

154-208 071130 Additional information (identity of vehicle used in 065021) was provided in this volume (Silva & Kishiyama, 2/7/90).

154-107 916532, "Teratology of Guthion," (Midwest Research Institute, 8/78). Technical Azinphos-methyl (no purity information) given by oral gavage at 0, 1.25, 2.5 or 5.0 mg/kg; 21-22 rats/group treated during organogenesis (days 6-15 of gestation), 14 rats/group treated from day 6 of gestation until pups weaned; nominal maternal NOEL = 2.5 mg/kg, nominal developmental toxicity NOEL \geq 5 mg/kg/day, insufficient number of litters for evaluation of the reproduction portion of the study; UNACCEPTABLE (missing individual data, no analyses of dosing solutions, no purity stated). (J. Christopher, 5/3/85).

EPA does not consider this a valid study (see 1986 EPA Re-registration Standard document).

TERATOLOGY, RABBIT

** 154-201 068778 "A Teratology Study in the Rabbit with Azinphos-methyl (Guthion Technical)," (Miles Inc., IN; Mobay Report No. 97406, 6/27/88). Azinphos-methyl, 87.7%

purity, administered daily by gavage at 0 (7% Emulphor), 1.0, 2.5, or 6.0 mg/kg/day to 17-18 pregnant American Dutch rabbits/group, on days 6-18 of gestation, with sacrifice on day 28. Dose selection based on range-finding studies. Maternal plasma and RBC cholinesterase activities measured on day 19 showed significant inhibition at 2.5 mg/kg (plasma) and 6.0 mg/kg (plasma, RBC); maternal brain cholinesterase activity also inhibited at 6.0 mg/kg (day 28). Maternal ChE NOEL = 1.0 mg/kg. No adverse effect. Developmental NOEL = 2.5 mg/kg (decreased litter size due to increased pre- and post-implantation loss). ACCEPTABLE. (Rinkus, 10/27/88).

154-221 087516 This volume contains additional data for study 154-201 068778 (historical control data of pre-sacral vertebrae). An EPA review of 068778 stated that “The developmental toxicity NOEL could not be established and historical control data are required.” EPA also responded, based on a review by Dynamac Corporation, with the statement: “A dose-related increase was observed in the incidence of fetuses and litters affected with lumbar and sacral vertebrae abnormalities (missing or extra arch; missing or extra centra) in the low- and mid-dose groups but not in the high dose group. Additional historical control data are required before the developmental toxicity of azinphos-methyl can be assessed.”

This rebuttal responds to Dynamac [contracted by EPA to review data] conclusions. Historical data requested by EPA were also provided (no worksheet). M. Silva, 2/7/90.

154-114 034198, “Studies for Embryotoxic and Teratogenic Effects on Rabbits Following Oral Administration,” (Bayer AG, 6/3/75, Bayer Report No. 5455, Mobay Report No. 44853). Azinphos-methyl (92.4%) by oral gavage at 0, 0.3, 1 or 3 mg/kg to 9-11 pregnant rabbits/group on days 6-18 of gestation. No adverse effects indicated. UNACCEPTABLE (no toxicity at top dose, no purity stated, missing uterine weights, corpora lutea and resorptions) - not upgradeable. (A. Apostolou, 9/11/85).

EPA does not consider this a valid study (see 1986 EPA Re-registration Standard document).

154-045 916534, “Short-Term Breeding Studies with Guthion in Rabbits,” (Tab. #18952), University of Chicago, 9/7/66). Azinphos-methyl (92.7% pure) at 0, 5, or 25 ppm on gestation days 8-15 in the diet, 10/group with 5 sacrificed at day 29 and 5 allowed to carry to termination. Insufficient information for independent adverse effects assessment. UNACCEPTABLE (missing diet analysis, food consumption, body weight and individual data), Not upgradeable (too few animals and fetuses, exposure period too short). (J. Christopher, 5/2/85).

EPA does not consider this a valid study (see 1986 EPA Re-registration Standard document).

TERATOLOGY, MOUSE

154-107 038720, “Teratology of Guthion,” (Midwest Research Institute, 8/78). Technical Azinphos-methyl (no purity information) was given at 0, 1.25, 2.5 or 5.0 mg/kg by oral

gavage on days 6-15 of gestation to 22-23 pregnant CD-1 mice. Report states that cholinergic signs were seen at 5 mg/kg/day but no data presented. No evidence of fetal toxicity or teratogenicity presented. Incomplete (missing individual data, purity information, no analyses of dosing solutions). UNACCEPTABLE. (J. Christopher, 5/31/85).

EPA does not consider this a valid study (see 1986 EPA Re-registration Standard document).

MUTAGENICITY, MISCELLANEOUS

154-143 038241 "The Mutagenicity in Prokaryotes of Insecticides, Acaricides and Nematicides," (publication in Residue Reviews 89 (1983): 129-178). Literature review of tests of azinphos-methyl (no purity information) on S. typhimurium, Serratia marcescens, E. Coli and B. subtilis. No adverse effects were indicated but insufficient information was provided for an independent assessment. Very Brief Summary (18 page review article). UNACCEPTABLE (one-liner only; no worksheet). (J. Gee, 8/31/87).

GENE MUTATION

154-114 034203, "Mutagenicity Evaluation of R 1582 (Azinphos-methyl) in the Reverse Mutation Induction Assay with Saccharomyces cerevisiae Strains, S128 and S211a," (Litton Bionetics, the Netherlands, 6/83) Azinphos-methyl (91.1%) at 0, 33.3, 100, 333.3, 1000, 3333.3 or 10,000 /ml to Saccharomyces cerevisiae strains S138 and S211a, single plates/dose level; No adverse effects indicated. UNACCEPTABLE (materials and methods were not adequately described) - possibly upgradeable. (Apostolou, 9/11/85).

154-131/114 011799, 034204 "Salmonella/Microsome Test for Determination of Point Mutations," (Mobay Report No. 69163, Bayer AG, Wuppertal-Elberfeld, 12/4/78). Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were tested with Azinphos-methyl (92.3%) at 0, 4, 20, 100, 500, 2500 /plate, 4 plates/dose level. UNACCEPTABLE (no individual plate counts, no positive controls without S9, no justification for not using a higher amount per plate). No increase in revertants. (J. Christopher, 5/2/85).

** 154-178 061361, "Salmonella/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test)." (Microbiological Associates, Report 94782 of Mobay, 8/5/87). Guthion, azinphos-methyl, 88.8% was tested with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 with and without rat liver activation, triplicate plates, two trials due to lack of satisfactory cytotoxicity in the first trial and an equivocal increase in revertants with TA100 only. First trial: 0, 33, 100, 333, 1000 and 2000 /plate; second trial: 0, 100, 333, 1000, 2000, 3333 and 4000 /plate. Equivocal effect

with TA100 was repeated up to 2000 /plate without activation and 1000 /plate with activation, then cytotoxic at higher amounts; increase did not meet the criteria of two- to three-fold increase usually accepted as showing a positive mutagenic response. ACCEPTABLE with no adverse effect clearly identified. (Gee, 6/21/88).

**** 154-203 069935, “E 1582 C. N. Azinphos-methyl Salmonella/Microsome Test to Evaluate for Point Mutagenic Effects” (Bayer AG, FRG; Mobay Report No. 97413, 5/6/88). Azinphos-methyl, 92.5% purity, tested with Salmonella typhimurium strains TA1535, TA100, TA1537, and TA98, without and with rat liver activation (two concentrations of S-9 fraction used), 4 plates/strain/dose, two trials, effectively covering the dose range of 0 to 9,600 /plate. Onset of cytotoxicity (decreased revertants/plate) observed at 1,200 /plate. No adverse effects otherwise identified. ACCEPTABLE. (Rinkus, 10/31/88).**

CHROMOSOME EFFECTS

154-131 011798, “Mutagenic Study with Guthion in Albino Mice,” (IBT No. E8921, IBT, 5/10/71). Dominant lethal. Azinphos-methyl at 0, 125 or 250 /kg to 12 male mice per dose level; judged to be Invalid in an audit by the registrant; insufficient information for adverse effects assessment; UNACCEPTABLE - not upgradeable. (J. Christopher, 5/2/85).

154-131/114 011800, 034205 “Micronucleus Test on Mouse to Evaluate R 1582 for Potential Mutagenic Effects,” (Mobay Report No. 69077, Bayer AG, Wuppertal-Elberfeld, 7/19/79). Azinphos-methyl (92.3%) was given by oral gavage at 0, 1.25, 2.5 or 5 mg/kg to 5 mice/sex/dose level, 2 doses at an interval of 24 hours, with mice sacrificed at 6 hours only (Schmid protocol); insufficient information for adverse effects assessment but no effect reported; UNACCEPTABLE (missing pilot study data, no clinical observations or pathology on mouse that died, doses not high enough with no signs of toxicity reported, unacceptable protocol), Not upgradeable. (J. Christopher, 5/2/85).

50615 (DPN Number) 001 052060, “Summary of the Results of the Negative Controls of the Micronucleus Test with Cremophor Suspensions After Single Oral Treatment and Preparation After 24 Hours,” (Mobay Report No. 90894). Negative control test results with 0.5%, 1.0%, 2.0% or 3.0% Cremophor suspension administered in a single oral treatment to mice (strain not stated). Dates of tests not provided. Supplemental to 131:011800, above.

154-131/114 011797, 034199 “Dominant Lethal Study on Male Mouse to Test for Mutagenic Effects,” (Mobay report No. 69076, Bayer AG, Wuppertal-Elberfeld, 6/7/79). Azinphos-methyl (92.3%) at 0 or 4 mg/kg in a single oral dose to 50 male mice/dose level; mated 1 male to 1 untreated female every 4 days for 12 matings, total of 598 females mated; insufficient information for adverse effects assessment but no toxicity or mutagenic effects reported, UNACCEPTABLE - Not upgradeable (single dose level with no data from the range-finding study, no positive control tested). (J. Christopher, 5/2/85).

**** 154-178 061360**, “E 1582 c. n. Azinphos Methyl Cytogenetic Study with Human Lymphocyte Cultures in Vitro to Evaluate for Harmful Effect on Chromosomes.” (Bayer AG, Germany, Report 94575, 10/20/86). Azinphos-methyl, 91.9%, was tested with human lymphocytes from one male and one female subject at 0, 1, 10 or 100 /ml for 24 hours without activation and at 0, 5, 50 or 500 /ml for 2.5 hours with rat liver S9 activation, followed by 21.5 hours further incubation. Two cultures per concentration per subject; scored 100 cells from each subject per concentration for aberrations and 4000 total for mitotic index from the preliminary, range-finding test. No increase in aberrations without S9 at any concentration but a several-fold increase in aberrations (excluding gaps), metaphases with exchanges and in metaphases with polyploidy at 500 /ml with activation in the presence of a mitotic index approximately 43% of control. No confirming repeat with activation. ACCEPTABLE. (Gee, 6/21/88).

**** 154-301 158335** “E 1582: Micronucleus Test on the Mouse,” by **B. Herbold**, Department of Toxicology, Bayer AG, Wuppertal, Germany (study #T 6058139; Bayer #106896; 3/7/95). **20 mice/sex were dosed *ip* with 5 mg/kg E 1582 (batch #230405033; 92.2% azinphos-methyl; a yellow coarse-grained powder) as an emulsion in 0.5% aqueous Cremophor. A negative control group of 5/sex were dosed with 0.5% Cremophor alone (10 ml/kg). A positive control group of 5/sex were dosed with 20 mg/kg cyclophosphamide. At 16, 24 & 48 hr after dosing 5/sex were euthanized (negative & positive controls were euthanized at 24 hr only; one treated group of 5/sex was present only to supply cells in the event of loss of other dosed animals), the bone marrow harvested from at least one femur/animal, smears prepared on coded slides, and the slides analyzed for micronuclei and for the polychromatic (PCE) to normochromatic (NCE) cell ratio. 1000 PCE=s/animal were scored. 4/40 treated animals died during the test period. The following clinical signs were observed for up to 6 hr: apathy, spasm, shivering, twitching, difficulty in breathing, salivation. Despite the ability of the positive control compound to increase the % micronucleated PCEs/1000/animal, no such ability was clearly forthcoming from the test article. Also, neither the positive controls nor the test article showed clear differences from controls in the PCE/NCE ratio. E 1582 is not considered to induce micronuclei in this system under the conditions tested. Acceptable. (Rubin, 12/24/97).**

DNA DAMAGE

154-114 034201, “POL Test on E. coli to Evaluate for Potential DNA Damage,” (Bayer AG, 2/22/84, Lab report. no. 12478, Mobay report No. 86471). DNA Damage/Repair; Azinphos-methyl (91.1%) at 0, 62.5, 1250, 2500, 5000, and 10,000 /plate, 4 plates/dose level, UNACCEPTABLE (no individual plate values, protocol inadequate in detail) - no cytotoxicity = No Test. (J. Remsen, 9/12/85).

**** 154-114 034202**, “Evaluation of R1582 C. N. Azinphos-Methyl in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay,” (Litton Bionetics, 11/83, LBI Project No.

20991, Mobay Report No. 86397). Azinphos-methyl at 0, 0.25, 0.5, 1.0, 2.5, 5.0, 10.1, 25.1, 50.3, or 100.5 /ml; precipitation at > 25.13 /ml, toxic at 50.3 /ml and higher; no adverse effects indicated; complete; ACCEPTABLE. (J. Remsen, 9/12/85).

NEUROTOXICITY (in hens)

**** 154-207 071143 Glaza, S.M., "Acute Delayed Neurotoxicity Study in the Domestic Fowl", (Hazleton Laboratories, project I.D. no. HLA 6232-101, 9/22/88). Guthion technical, purity 85%, was administered by a single gavage at 330 mg/kg (unprotected LD50) to the crops of 30 white leghorn hens, with treatment repeated on survivors 21 days later. The study included untreated, vehicle (corn oil), and positive (TOTP) controls (10 hens/group). Animals were observed for a total of 44 days. No adverse effect (no evidence of delayed neurotoxicity was observed). Originally not acceptable (analyses of dosing material and statistical analyses for histopathology were missing from the report; there were several inconsistencies in the data). Upgraded to acceptable status on review of corrected report in 244:088571 (see below). (Kishiyama & Silva, 2/9/90; Aldous, 12/27/90).**

154-244 088571 [Addendum to 207:071143]. This re-submission supplies the three items which were noted as deficient in the original review: analysis of test article (demonstrating appropriate content and stability), a summary table of statistical analyses, and correction of discrepancies about time of death for several animals. These additional data permit an upgrade of the study to acceptable status. Aldous, 12/27/90.

154-100 916524, "Ethyl-Gusathion, Subchronic Neurotoxicity Tests on Chickens," (Tab #24063, Bayer AG, Wuppertal - Elberfeld, 12/18/68). Ethyl-gusathion, 50% in Silkasil S; 0, 75, 150, 300 or 600 ppm to 8 hens in the diet for 30 days, followed by 4 weeks of observations; incomplete (missing individual data, diet analysis), UNACCEPTABLE - Not upgradeable (test material tested was not azinphos-methyl but azinphos-ethyl). (J. Christopher, 5/2/85).

154-045 024182. [See also record. #916461 (Tab #017879) for histological findings]. "Neurotoxic Studies with Guthion Active Ingredient," (Tab #17563, Bayer AG, Wuppertal - Elberfeld, 5/20/65). Azinphos-methyl (80%) at 0, 900, 1200, 1500 or 1800 ppm in the diet of hens for 30 days, followed by 4 weeks observations; Insufficient information for adverse effects assessment; incomplete (no individual data), UNACCEPTABLE - not upgradeable (no positive control, a.i. probably not absorbed, inappropriate route of administration). (J. Christopher, 5/2/85).

154-045 024541, "Report on Demyelination Studies on Hens, Guthion," (Tab #15949, Harris Lab., 3/12/65). Azinphos-methyl (no purity stated) at 0, 10, 50 or 100 ppm in the diet to 6 hens/group for 30 days followed by 30 days of observations; insufficient information for adverse effects assessment; incomplete (no individual data, no clinical observations), UNACCEPTABLE - not upgradeable (no positive control, inappropriate route of administration). (J. Christopher, 5/2/85).

154-045 024183, “Neurotoxic Studies with Guthion Active Ingredient,” (Tab #15221, Bayer AG, Wuppertal - Elberfeld, 11/30/64). Azinphos-methyl (diluted to 80%) at 0, 75, 150, 300 or 600 ppm in the diet of hens for 30 days followed by a 4-week observations period; Insufficient information for adverse effects assessment; incomplete (missing clinical observations or histopathology data), UNACCEPTABLE - not upgradeable (no positive control, inappropriate route of administration). (J. Christopher, 5/2/85).

NEUROTOXICITY (in rats, various durations)

**** 154-269 132080 Sheets, L. “An Acute Oral Neurotoxicity Screening Study with Technical Grade Azinphos-Methyl (GUTHION7) in Fischer 344 Rats” (Miles, Inc., Agriculture Division, Toxicology, Stilwell, Kansas. Miles Report #106365, 8/29/94). Azinphos-methyl (purity 92.2-92.8%, Batch #3030050) was administered by single oral gavage to 18 Fischer 344 rats/sex/dose at 0, 2, 6 and 13 mg/kg for males and 0, 1, 3 and 6 mg/kg for females. (one-liner, control.) Five high-dose males and 15 high-dose females died on the day of exposure. Clinical signs associated with cholinesterase (ChE) inhibition were seen mostly on day 0 and included muscle fasciculations, oral stain, urine stain, nasal stain, tremors and uncoordinated gait in high-dose males (fewer signs reported in females because of high mortality). NOEL (clinical signs) = 2 mg/kg (males), 3 mg/kg (females). FOB findings (day 0) in mid- and high dose groups included gait incoordination, repetitive chewing, muscle fasciculations, tremors, lacrimation, salivation, minimal open-field movement, reduced touch responses, uncoordinated righting response, decreased body temperature and decreased grip strength. NOEL (FOB) = 2 mg/kg (males) and 1 mg/kg (females). Motor and locomotor activity was decreased in mid- and high-dose males and high-dose females (some high-dose rats showed decreases in activity on day 7). NOEL (motor activity) = 2 mg/kg (males) and 3 mg/kg (females). No compound-related changes in gross- or micropathology or organ weights. **No Adverse Effects.** ACCEPTABLE. Kellner and Gee, 10/24/94.**

Note: An adverse effects disclosure in accordance with the reporting requirements of FIFRA 6(a)(2) was submitted with respect to study 154-269:132080 (Miles report # 106365). The adverse effects in question were reduced RChE and PChE activity in the low-dose (2 mg/kg) males and reduced RChE activity in low-dose (1 mg/kg) females (i.e., no NOEL for ChE inhibition). The disclosure was sent to the reviewing agencies after the final report (disclosure was dated 9/27/94 and the study was submitted on 9/7/94). According to the accompanying cover-letter, the delay was due to the urgency of submitting this study to meet the U.S. EPA Data Call-In requirements. No new data were presented and no volume or record number was assigned to this submission (Tracking ID SBC-149131-EA). No Worksheet. Kellner, 11/1/94.

**** 154-277 135548 Sheets, L.P., “A subchronic dietary neurotoxicity screening study with technical grade azinphos-methyl (Guthion®) in Fischer 344 rats”, Miles, Inc., Agricultural Division, Toxicology (Stilwell, Kansas), 2/14/95. Miles Report No. 106839. Eighteen rats/sex/group were dosed with azinphos-methyl (92.4%) at 0, 15, 45, or 120 ppm (males) or**

0, 15, 45, or 90 ppm (females) for 13 weeks. Six/sex/group were monitored for RBC and plasma cholinesterase at weeks 0, 4, and 13. Also, brain cholinesterase was measured in these rats at termination. The remaining 12/sex/group were subject to evaluations of motor activity and FOB evaluations. At termination, 6/sex/group were perfused and prepared for microscopic evaluation. Only controls and high dose animals were so examined, since there was no high dose histopathological effect. No cholinesterase inhibition NOEL was determined: dose-related inhibition was observed in brain, RBC, and plasma cholinesterase over the range tested. A conservative NOEL for findings other than cholinesterase enzyme assays was 15 ppm, based on slightly increased reactivity and on a general increase in incidence or degree of urine-stained coat in females. Common findings at 90 to 120 ppm included increased reactivity, coat stains (especially perianal stains in males, and urine stains in females), body weight decrements, and a decrease in motor and locomotor activity. Also, findings primarily or exclusively in 90 ppm females included uncoordinated gait, tremor, uncoordinated righting reflex, reduced diet intake, and a decrement in forelimb grip strength. Study is acceptable, with no adverse subchronic neurotoxicity indicated. Acute/subacute cholinesterase-related effects are relevant to safety assessment. Aldous, 5/30/95.

NOTE: A WORKSHEET HAS BEEN PROVIDED FOR A REPORT CONTAINING METHOD VALIDATION INFORMATION, BELOW. THIS REPORT WAS SUBMITTED IN SUPPORT OF ANOTHER MILES PRODUCT, SULPROFOS. THE ONE-LINER FOLLOWS.

374-087 122985 Sheets, L.P., "Historical control and method validation studies in rats for the acute and subchronic neurotoxicity screening battery", Miles Inc., Agricultural Division, Toxicology, Stilwell, Kansas, 3/31/93. Miles Report No. 103979. Motor activity evaluations were done for triadimefon and chlorpromazine, FOB data were obtained for acrylamide and carbaryl, and microscopy was done for acrylamide and trimethyltin studies on rats. The studies presented validate the investigators' capability to produce valid rat acute to subchronic duration neurotoxicity studies. Typical tables are included in the review as reference positive and negative control data. Data apply toward method validation for rat neurotoxicity studies of at least azinphos-methyl, sulprofos, disulfoton, and methamidophos. Aldous, 6/29/95.

DEVELOPMENTAL NEUROTOXICITY

(includes supplementary studies on age differences in cholinesterase inhibition sensitivity)

154-0321 210175 Sheets, L. P., "A developmental neurotoxicity screening study with technical grade Azinphos-methyl (Guthion®) in Wistar rats," Bayer Corp., Stilwell, KS, 6/20/02, Study # 01-D72-C1. Bayer Report No. G200115. Thirty mated Wistar Hannover [CrI:WI(Glx/BRL/Han) IGS BR] dams/group were dosed in diet with 0, 3, 10, or 15 ppm azinphos-methyl (90.6% purity) throughout gestation and lactation (ending lactation day 21). Estimated mean gestation exposures were 0.2, 0.8, and 1.1 mg/kg/day. Corresponding mean

exposures during lactation were 0.4, 1.4, and 2.0 mg/kg/day for days 0-7; 0.5, 1.9, and 2.9 mg/kg/day for days 7-14; and 0.6, 2.1, and 3.2 mg/kg/day for days 14-21. At least 20 litters per group were of sufficient size to maintain offspring until sacrifice at about postnatal day (PND) 75. Maternal NOEL = 3 ppm (19% and 48% reductions in brain acetylcholinesterase at 10 and 15 ppm, respectively). Offspring NOEL is not yet determined, due to a possibly treatment-related increase in retinal dysplasia in 15 ppm males at PND 75 terminal sacrifice, without adequate basis to consider this as incidental. Many endpoints were evaluated in intermediate dose levels, and no treatment effects were observed at 3 or 10 ppm. Study is not acceptable. DPR requests control incidences of retinal dysplasia, and microscopic evaluations of eyes in intermediate groups in this study. Validation studies cited in the report as Refs. 8, 9, 12, and (if relevant) Ref. 19 are requested. Investigators opted to eliminate the PND 11 sacrifice of pups with brain morphometric measurements and histopathology, and substituted a PND 21 sacrifice for those parameters, citing a former EPA approval to do so for a distantly related material (tribufos). A justification for this change of procedure in the present case is requested. Clarification is requested on statistics used for morphometric measurements, with corrections provided if indicated. Details on DPR concerns on report adequacy are in the review discussion section. Other than these issues, this study addressed the full scope of evaluations that pertain to developmental neurotoxicity studies. Aldous, April 6, 2004.

154-0320 210174 Sheets, L. P. "Cholinesterase inhibition in young-adult and neonatal (11-day-old) Wistar rats treated by gavage with a single dose of technical grade Azinphos-methyl (Guthion®)," Bayer Corp., Stilwell, KS, 12/19/03. Bayer Report No. G200737. A time-course study found near-maximal cholinesterase inhibition responses at about 0.75 hr in neonates (PND 11) and adults. This time point was used for subsequent dose-response tests. NOEL's for inhibition were nominally 0.5 mg/kg for both PND 11 pups and adult rats. There was statistically significant brain, RBC, and plasma cholinesterase inhibition in PND 11 pups of both sexes at 1 mg/kg. A marked (47%) inhibition of RBC cholinesterase in adult males was the only statistically significant finding in 1 mg/kg adults. Plasma, RBC, and brain cholinesterases were significantly inhibited at 2 mg/kg in adult males, and RBC cholinesterase was significantly inhibited in 2 mg/kg adult females. Investigators considered results to show comparable responsiveness at both ages. DPR review concludes that the data suggest about a 2-fold greater sensitivity in neonatal rats. This considers the greater percentage cholinesterase inhibition found in the time course comparisons, in which inhibition following 2 mg/kg dosing to PND 11 pups was compared to adult responses at 3 mg/kg (F) and 6 mg/kg (M). Further, peak response time in the neonates appears to be slightly longer than in adults. Brain cholinesterase inhibition in dose-response studies also indicated about a 2-fold increased sensitivity in neonates. Control brain cholinesterase levels at PND 11 were about 44% of adult levels. Supplementary data. Aldous, 4/7/04.

154 - 0322 210505 Sheets, L. P. "Cholinesterase inhibition in young-adult and neonatal (11-day-old) Wistar rats after repeated exposure by gavage to technical grade azinphos-methyl (GUTHION®)." (Bayer CropScience LP, Stilwell, KS, study numbers 02-N12-MX (adults, October 2002) and 03-D12-TH (neonates, November 2003), Bayer Report No. 200989, March 29, 2004) Adults were 8 - 10 weeks at start of exposure and neonates were exposed on PND 11 through 21 (preweaning). For adults, there were 6/sex/group given analytical doses of technical

grade azinphos-methyl (90 - 92% purity) of 0 (0.5% methylcellulose and 0.4% Tween 80 in water), 0.25, 0.54, 1.0 or 1.6 mg/kg/day for 11 consecutive days. Neonates (10 - 12/sex/dose) were given doses of 0, 0.24, 0.51 or 1.0 mg/kg/day, in 10 ml/kg. The neonates were from litters bred for that purpose. Only litters with 8 pups (4/4 or 5/3 by sex) were used and 1/sex/litter was distributed in each of the four dose groups, when possible. Pups were kept with the dam until day 21. Body weights were taken prior to dosing to determine the volume of dosing suspension per animal but weights were not reported. The only parameters reported were cholinesterase activities for plasma, RBC and whole brain. Based on previous studies, the time of peak effect of azinphos-methyl on cholinesterase of adults was 45 - 50 minutes after dosing, so these animals were sacrificed and sampled during that period after the last (11th) dose. For neonates, the time of peak effect lasted from 45 - 50 minutes to 3 hours following treatment so they were sampled at 90 ± 10 minutes after the last dosing. Cholinesterase was assayed by a modified Ellman method, using 6,6'-dithio-dinicotinic acid with absorbance at 340 nm. In adults, doses of 0.25 and 0.54 (0.50 nominal) mg/kg did not effect any ChE measurement with a LOEL = 1.0 mg/kg, based on significantly lower activity in RBC ChE of both sexes (M: 41%, F: 53%). At 1.5 mg/kg, plasma, RBC and brain were all significantly affected in both sexes with no clear gender bias. In neonates, RBC ChE was significantly lower in males and brain was significantly lower in both sexes at 0.25 mg/kg (M: 9%, F: 8%, both statistically significant at $p \leq 0.05$.) At 0.5 mg/kg, all three ChE measurements were significantly lower in both sexes. NOEL < 0.25 mg/kg and LOEL = 0.25 mg/kg in neonates given 11 consecutive doses. Supplemental study. (Gee, 10/19/04) NOTE: Record 210505, Bayer Report Number 200989, was submitted as a 6(a)(2) report. Due to the nature of the study, no adverse effect was specifically associated with the results. (Gee, 10/19/04)

NEUROTOXICITY (human subjects)

154-318 182478 McFarlane, P. and S. Freestone, AA randomized double blind placebo controlled study with Azinphos-methyl to determine the no effect level on plasma and RBC cholinesterase activity after repeat doses, Inveresk Research (Tranent, Scotland), 4/15/99. Laboratory Project ID: ICR 013580. Twelve healthy men (aged 18-50 yr, non-smokers) were randomly assigned to either placebo (N = 4) or treatment group (N = 8). Study was under auspices of an Independent Research Ethics Committee. All subjects received one gelatin capsule daily [0.25 mg/kg/day Azinphos-methyl (91.6% purity) for treated men, or lactose for the placebo group] for 28 days. Subjects resided in the clinic during the entire study, under “constant medical and nursing supervision,” “received a standardized diet.” **They were evaluated for vital signs daily just before dosing and 24 hr after the last treatment. EKG=s** were obtained before dosing on days 1, 7, 14, 21, and 28. Blood and urine samples were taken on the same schedule for hematology, clinical chemistry, and urinalysis. In addition, plasma and RBC cholinesterase assays were performed on blood samples taken on pre-study days -14, -12, -10, -8, -6, -4, -2, and -1, as well as daily before each dose, and 4 hr post-dose on days 1, 2, 3, 4, 5, 7, 10, 14, 17, 21, 24, and 28. All observed or reported “adverse” events **were recorded. NOEL = 0.25 mg/kg/day (no treatment effects at the only dose tested). Study does not fill data requirements, but provides valid data for these parameters. No adverse effects. Aldous,**

11/6/01.

154-308 167613 McFarlane, P. and S. Freestone, AA randomised double blind ascending single oral dose study with Azinphos-methyl to determine the no effect level on plasma and RBC cholinesterase activity, Inveresk Research International, Tranent, Scotland, 12/21/98. Laboratory Project ID: ICR 013219. A copy of a memorandum from Jay Schreider dated 3/31/99 is found in the front section of this volume. This memo considered the study appropriate for use in risk assessment. No plasma or RBC inhibition was found at oral (gelatin capsule) dose levels as high as 0.75 mg/kg in either sex of adult subjects. An adjunct group of males was also tested at 1.0 mg/kg, similarly negative for cholinesterase inhibition. None of the other measured parameters [comparable to those evaluated in the subsequent repeat-dose study (154-318 182478)] indicated adverse effects. The DPR review describes in detail the steps taken to ensure the safety of the subjects and to meet the standards of the Independent Research Ethics Committee. A key to subject safety was review of results of each completed group prior to advancing to a higher dose level.

154-305 163501 protocol for 154-308 167613, above.

WORKER EXPOSURE STUDIES

154-307 167193 Selim, S., "Absorption, excretion, balance and pharmacokinetics of ¹⁴C Radioactivity after single dose dermal application of three dose levels of ¹⁴C labeled Guthion to healthy volunteers," 2/17/99. XBL Study No. 98052. This study was reviewed by T. Thongsinthusak (DPR Worker Health and Safety Branch) on April 7, 1999. He concluded that "An average dermal absorption value of 21.5% is recommended for use in the calculation of absorbed dose of Azinphos-methyl."

154-306 165488 This is a 5/7/98 draft of 154-307 167193, above.

154-304 160553 Protocol for 154-307 167193, above.

154-285 141713 Stokes, L., A. Stark, E. Marshall, and A. Narang, "Neurotoxicity among pesticide applicators exposed to organophosphates," *Occup Environ Med* **52**:648-653 (1995). An epidemiology study in which a cohort of 90 male apple orchard applicators from New York State were evaluated to determine if short-term exposure to azinphos-methyl produced acute health effects. The applicators were first questioned off season and then again during the spraying season for the presence of several acute signs and symptoms. Short-term exposure was validated by measuring dimethylthiophosphate in the urine. Chronic signs of peripheral nerve damage were determined by vibration sensitivity thresholds in both hands and feet during the off season. Long-term exposure to pesticides was determined by questionnaire. Seventy-eight applicators (86%) had used azinphos-methyl during the previous two growing seasons. The mean number of

years azinphos-methyl had been used by the applicators was 14 years. The average number of applications per season was 5 times. Of the acute signs and symptoms related to organophosphate poisoning, only headaches were more frequent during the spraying season than off. The mean vibration threshold scores for the hands were significantly higher for applicators when compared with scores for the population based controls matched on age, sex, and county of residence. (Above paragraph from preliminary July 2000 DPR draft of report: "Evaluation of Azinphos-methyl as a toxic air contaminant," by **C. Lewis *et al***).