

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
FOSTHIAZATE (formerly IKI-1145)

Chemical Code # 2325, Document Processing Number (DPN) # 51746
SB 950 # N/A

Original date: April 4, 1991
Revisions: 6/14/93, 5/29/08, 6/9/08

I. 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, possible adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, possible adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	No data gap, possible adverse effect

Toxicology one-liners are attached.

All record numbers for the above study types through 221588 (Document No. 51746-0064) were examined. This includes all relevant studies indexed by DPR as of 3/27/08.

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: t20080609.wpd

Revised by Aldous, 5/29/08 and 6/9/08.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

****51746-0025 143110** Aughton, P., "IKI-1145: Combined chronic toxicity and carcinogenicity study by dietary administration to CD rats for 104 weeks," Life Science Research, Ltd. (Eye, Suffolk, England), Oct. 9, 1990. LSR Report #: 89/ISK089/0557. Test article was Fosthiazate (IKI-1145 Technical), purity 93.3%, Lot No. 8603. Groups of 50 CD rats/sex/group were assigned to the oncogenicity study at 0, 1, 10, 50, and 200 ppm (adjusted for a.i. purity) for 104 weeks. Chronic study phase groups (10/sex/group) received the above diets for 52 weeks. Estimated average achieved dosages of a.i. for the 2-year study, corrected for purity, were 0.039, 0.38, 1.94, and 8.34 mg/kg/day for males, and 0.051, 0.50, 2.45, and 11.69 mg/kg/day for females. NOEL for circulating cholinesterase enzyme inhibition was 1 ppm, based on marked, dose-related decreases in blood cholinesterases [plasma acetylcholinesterase (AChE), plasma butyrylcholinesterase (BuChE), and RBC AChE] at 10 to 200 ppm. Brain AChE was profoundly affected in both sexes: inhibition was dose-related at 50 and 200 ppm, but absent at 10 ppm. A working NOEL for findings other than ChE inhibition was 10 ppm, based on foamy interstitial cells of the ovaries in 50 and 200 ppm females, and on modest reductions of RBC parameters in both sexes (RBC count, Hb, and HCT) at 50 and 200 ppm. Neutrophil counts were commonly elevated at 200 ppm. Reticulocyte counts were statistically elevated at 50 to 200 ppm in males and at 10 to 200 ppm in females. Considering the limited response in RBC parameters throughout the dose range tested, it is doubtful that these reticulocyte values represent a key finding. There were several common or definitive findings at 200 ppm. Both sexes at that dose showed high incidences of changes in the adrenal cortex (vacuolation, zona fasciculata) and pituitary (vacuolation, pars nervosa) in chronic and oncogenicity groups. Vacuolation of the adrenal zona glomerulosa was additionally observed at 1 year in 200 ppm males and females, but not in lifetime rats. There were general increases in retinal atrophy in lifetime study rats at 200 ppm in both sexes, and lesser but plausibly treatment-related increases in cataracts in 200 ppm females. Incidence of degenerative myopathy of skeletal muscle (usually "minimal" to "slight" degree) was significantly elevated in 200 ppm males and females. There was no definitive body weight effect in either sex, although high dose females body weights fell behind other groups near study termination. Food consumption and clinical signs data likewise did not indicate excessive general toxicity at 200 ppm. No oncogenicity was observed. Study is **acceptable**, with brain AChE inhibition as a "possible adverse effect." Aldous, May 1, 2008.

51746-0059 221512 Killeen, J. C., "Statistical analysis of week 12 blood cholinesterase activity in a chronic rat study with technical fosthiazate," Submitter's Document No. 6610-95-0266-TX-001, Dec. 8, 1995. This record notes that the statistical evaluation of Week 12 cholinesterase data in the interim report (see Record No. 90684, below) used parametric data analytical techniques, whereas the final report (Record No. 143110, above) used non-parametric analyses. The author notes that the criteria for parametric analyses were not met (normal distribution, homogeneity of data), so that non-parametric analyses were appropriate. This record was submitted in response to a U.S. EPA review (DER included in this record). DPR reviewer Aldous confirmed that in the instance of control and 1 ppm BuChE values in females at

12 weeks (which was reported as statistically significant in the interim report, but not in the final report), variances were unequal ($p = 0.003$). This report validates procedures used in the final report reviewed above. Useful supplementary data (no DPR worksheet). Aldous, Feb. 8, 2008.

51746-014; 90684; "IKI-1145: Combined Chronic Toxicity and Carcinogenicity Study by Dietary Administration to CD Rats for 104 Weeks (Interim Report: 0-52 Weeks)." Interim data were reviewed by T. Moore on 4/12/91. Final study is Record No. 143110, above.

CHRONIC TOXICITY, RAT

(See Combined, Rat, above)

CHRONIC TOXICITY, DOG

**51746-0024 143108 Tauchi, K., "Chronic oral toxicity study on the dog treated with IKI-1145 technical for 12 months," Toxicology Research Center, Imamichi Institute for Animal Reproduction, Dejima-mura, Niihari-gun, Ibaraki, Japan, Aug. 5, 1991. Study # 230. Five beagles/sex/group were dosed daily by capsule with 0, 0.05, 0.1, 0.5, or 5 mg/kg/day of Fosthiazate (IKI-1145 Technical), purity 93.8%, in a chronic study. NOEL for findings other than cholinesterase inhibition = 0.5 mg/kg/day, based on the following responses at 5 mg/kg/day. Histopathology findings at 5 mg/kg/day included increased incidences and/or severity scores of hypertrophy of the adrenal cortical zona fasciculata and of the zona glomerulosa. Both sexes had increased pallor of zona glomerulosa cells. Males had increased pallor of the zona fasciculata. Vacuolization (minimal severity) was observed in zona fasciculata in 3 high dose males and in 1 high dose female, but not in any other groups, suggesting a possible minimal response. Hematology findings of mild anemia, primarily in males, were based on slight reductions in RBC parameters in both sexes (HCT, RBC count, and Hb), plus a modest platelet increase in males and modest increase in reticulocyte counts in females). Cholinesterase inhibition was clearly evident in both sexes at most sampling periods for plasma acetylcholinesterase and for plasma butyrylcholinesterase at 0.5 to 5 mg/kg/day, with non-significantly lower activities for these parameters in 0.1 mg/kg/day males (plausibly treatment-related). Acetylcholinesterase activities in RBC and brain were not affected at any dose levels in either sex. Acceptable (not accepted prior to 6/9/08 review for lack of documented weekly physical examinations: evidence that dogs received careful examinations was provided as supplementary information on June 4, 2008). No adverse effects indicated. Aldous, 5/28/08, amended June 9, 2008 (see following paragraph).

Re-evaluation of Document No. 51746-0024, Record # 143108 (the primary dog chronic study) for Fosthiazate suggests that the study should be re-classified as **acceptable**, based on (1) evidence that the detail of daily findings provided information comparable to what might have been provided in weekly formal physical examinations and (2) the subchronic study for this compound (51746-013 90683) included veterinary and neurological examinations after 12 weeks at a comparable dosing regimen (which were negative). Chronic and subchronic studies identify the same well-defined histopathology changes at 5 mg/kg/day, and it is clear that cholinesterase inhibition defines the most sensitive NOEL for this compound. Aldous, 6/6/08.

NOTE: see DPR Document No. 51746-0055, Record No. 221508, for a Pathology Working Group (PWG) peer review of adrenal cortex histopathology. In between the times of the first report of this study and the report of the PWG, the adrenal slides were examined twice, with different conclusions about the NOEL (see especially DPR Document No. 51746-0053, Record No. 221506). The PWG analysis by 5 pathologists of both chronic and subchronic study adrenal gland slides of all dogs in both studies was designed to settle the differences between previous evaluations, and forms the basis of the NOEL and descriptions of adrenal cortex histopathology described above.

51746-0060 221513. This is an exact duplicate of 51746-0055 221508. Aldous, 2/8/08.

51746-0055 221508 [Supplemental to: Document # 51746-0024, Record # 143108, Sponsor's Study No. 230]. "Chronic oral toxicity study on the dog treated with IKI-1145 Technical for 12 months: Pathology Working Group Peer Review (Imamichi Study No. 230)." Sept. 1, 1995. This record has two parts, each concerning PWG re-evaluations of adrenal tissue slides of dog studies. The part relating to the dog chronic study begins on page 150. The PWG was comprised of 5 pathologists refereed by Dr. Jerry F. Hardisty of Experimental Pathology Laboratories, Inc. (EPL). They conducted a "blind" examination of coded adrenal gland slides from all dogs on study. Findings of the PWG for adrenal histopathology are considered as the definitive diagnoses for adrenal histopathology, and are found in the 1-liner of the primary report of Record # 143108. In the present record, diagnoses of each adrenal cortex slide by the PWG were compared with three earlier evaluations (i.e. by the original study pathologist, by a pair of ISK pathologists, and by a single EPL pathologist). The DPR review of this record includes comments on this cross-comparison. Aldous, 2/13/08.

51746-0053 221506 This report describes the evaluation of dog chronic and subchronic study adrenal gland slides by Dr. D. A. Banas [the EPL reviewing pathologist who reviewed these slides prior to the evaluation by the Pathology Working Group (PWG), as described above for Record No. 221508]. Pages 47 and 48 of this report crisply summarize the previous examination of the slides by the ISK reviewing pathologist for subchronic and chronic dog studies, respectively. Both of these diagnoses were incorporated into the PWG report, and acknowledged in the associated DPR reviews. No separate worksheet is needed for this record. Aldous, 2/13/08.

51746-0027 143113 This is an earlier submission of the short report designated by DPR as 51746-0053 221506, lacking a few introductory pages found in Record No. 221506. No DPR worksheet. Aldous, 2/13/08.

ONCOGENICITY, RAT

(See Combined, Rat, above)

ONCOGENICITY, MOUSE

**51746-0026 143112 Aughton, P. "IKI-1145: carcinogenicity study by dietary administration to CD-1 mice for up to 104 weeks," Life Science Research, Ltd. (Eye, Suffolk, England), Oct. 9,

1990. LSR Report #: 89/ISK088/0624. Sixty CD-1 mice/sex/group were dosed in diet with Fosthiazate (IKI-1145 Technical, Lot # 8603, purity 93.3%) at 0 (2 groups of 60/sex), 10, 30, 100, or 300 ppm for 102 weeks. Estimated achieved doses of fosthiazate (corrected for purity) were 1.02, 3.10, 10.3, and 30.5 mg/kg/day for males, and 1.11, 3.20, 10.4, and 39.2 mg/kg/day for females. NOEL = 30 ppm (3.20 mg/kg/day) in females, based on pigmentation in the cortico-medullary region of the kidney. NOEL for males was 100 ppm (10.3 mg/kg/day). Common findings at 300 ppm (in both sexes unless specified) included body weight reduction (21% below controls in M and 31% below controls in F at termination); 14% food consumption reduction in males; general increase in mortalities in 300 ppm females during the 3rd quarter of the study (equivocal treatment effect: no clear rationale for this difference); significant increase in adrenal absolute and relative weights in 300 ppm females; adrenal pathology consisting of greatly increased pigmentation in the cortico-medullary region, with mineralization of affected cortical cells in many cases; papillary mineralization in kidneys; and vacuolation of the pituitary pars nervosa. Treatment did not induce tumors. The high dose exceeded body weight decrement criteria for an MTD in both sexes. Males at this dose had significantly **reduced** incidences of both hepatocellular adenomas and carcinomas, possibly associated with reduced food consumption and/or body weight. The placement of the next-highest dose assured a valid dose-response assessment in a sustainable dose range. Acceptable, with no adverse effects. Aldous, 4/28/08.

REPRODUCTION, RAT

****51746-016 090688** "IKI-Technical: Reproductive Performance Study in Rats Treated Continuously Through Two Successive Generations," Rat; 834; Fosthiazate (IKI-1145 Technical), Lot # 8603; Life Sciences Research Ltd, Suffolk, England; LSR Report 89/ISK109/0923; 11/30/89. In response to the U.S. EPA DER of 6/20/90 (51746-0032 221439), EPA and DPR received supplementary information in two small reports: 51746-061 221514 (dated 8/26/97), Lucas, F., "A rebuttal to an EPA review of the study entitled 'IKI-Technical: Reproductive Performance Study in Rats Treated Continuously Through Two Successive Generations,'" Submitter's Document No. 7081-97-0148-TX-001; and 51746-062 221515 (dated July 12, 1999) Reiss, R. and C. Valdez-Flores, "Statistical reanalysis of 'IKI-Technical: Reproductive Performance Study in Rats Treated Continuously Through Two Successive Generations,'" (no submitter's report number). DPR re-examination considering the above submissions follows. Dietary exposure to 0, 3, 10, 30, 100 ppm Fosthiazate (formulations adjusted for a.i. content: 93.4% purity). Twenty-five SD rats/sex/group, two generations (100 ppm group was not continued as F1 generation parents due to excessive toxicity). Systemic parental NOEL = 10 ppm (slight increase in adrenal weights in F1 females, associated with a slight increase in hypertrophy of the adrenal cortical zona glomerulosa). Reproductive NOEL = 30 ppm (increased pre-coital interval, and increased duration of gestation). Offspring growth and viability NOEL = 30 ppm (high pup mortality, and marked pup body weight decrements compared to controls throughout gestation). Additional adult systemic effects at 100 ppm included marked increase in hypertrophy of the zona glomerulosa of the adrenal cortex, and foamy appearance of ovarian interstitial cells. **Possible adverse effects:** (primarily due to pup viability and growth effects, above). Study **acceptable**. (Moore, 12/26/90; re-examined with above supplementary data by Aldous, 5/29/08).

TERATOLOGY, RAT

** 51746-021; 119179, 119222; “IKI-1145: Teratology Study in the Rat (Final Report)” (author: Willoughby, C.R., Life Science Research, Ltd., Suffolk, UK, LSR Report # 90/ISK123/0068, 10/23/90); 833; IKI-1145 technical (93.3% purity, Lot 8603); 0, 3, 5, and 10 mg/kg/day in 0.5% methylcellulose to 24 pregnant CD rats/dose on day 6 to day 15 of gestation; **no adverse effects**; dams receiving 10 mg/kg/day exhibited reduction in body weight gain (91.4% of control, $P < 0.001$) without any changes in food and water consumption; no treatment-related effects in litter parameters; growth and development of fetuses in utero were not affected; three fetuses, one at the low dose and two at the mid dose showed double diaphragmatic hernia, but was not considered to be treatment-related due to lack of dose-response relationship; **developmental NOEL \geq 10 mg/kg/day** (no effect at HDT); **Maternal NOEL = 5 mg/kg/day** (decreased body weight gain first reported on day 9); **acceptable**; (Leung, 6/9/93).

TERATOLOGY, RABBIT

51746-0015 090686 Bailey, G. P., “IKI-1145: Teratology study in the rabbit,” Life Science Research, Ltd. (Eye, Suffolk, England), 12/15/89. LSR Report No. 89/ISK118/0694. Fifteen inseminated NZW does per group were dosed by gavage in a developmental toxicity study with a 0.5% w/v aqueous methylcellulose suspensions of Fosthiazate (IKI-1145 Technical) (purity 93.3%) at 0, 0.5, 1.0, 1.5, and 2.0 mg/kg/day. Maternal NOEL = 1.5 mg/kg/day [based on dose-response for maternal deaths and moribund sacrifices in the associated pilot study (Record No. 090685)]. Developmental NOEL < 0.5 mg/kg/day (no change from earlier review). Basic maternal and developmental endpoints, including mean fetal weight, were unaffected. There was one fetus in each of the treatment groups with major malformations, prompting the original Medical Toxicology Branch reviewer to attribute findings to treatment, and to determine that the study had no NOEL for developmental toxicity. Findings were: a 0.5 mg/kg/day fetus with “cleft lip, distance between eyes reduced, and eyes displaced cranially,” and also “brow ridge.” A 1.0 mg/kg/day fetus had a “proboscis, eyes displaced towards mid-line, upper jaw misshapen, and gastroschisis.” A 1.5 mg/kg/day fetus had an “absence of olfactory lobes, head appears slightly narrowed between eye orbits, cerebral hemispheres appear to be continuous throughout whole length. Moderate/severe internal hydrocephaly.” An additional fetus from another 1.5 mg/kg/day doe had anomalous ribs: “right ribs displaced caudally by 1 vertebra; no right rib on 1st thoracic vertebra and additional floating right 12th rib on 12th thoracic vertebra.” A 2.0 mg/kg/day fetus had “acrania, bilateral fore-limb flexure, 2nd digit of both fore-limbs shortened with claw missing, malrotation of hind-limbs, body length apparently reduced, tail kinked, lungs not inflated, aortic arch reduced, stomach contents reduced, kidneys slightly displaced caudally, and major vessels to kidneys apparently reduced.” The same fetus was described under skeletal findings as grossly abnormal, with “Acrania, no skeletal structure above exoccipitals and fragments of basioccipital. 12th pair of ribs rudimentary. Agenesis of centra of cervical vertebrae and 1st to 5th thoracic vertebrae. 6th and 7th thoracic vertebral centra bipartite, 8th to 10th cleft. 1st and 2nd lumbar vertebral centra cleft, right hemicentrum of 3rd reduced. Agenesis of centra of 4th lumbar to 2nd sacral vertebrae. Arches of 1st sacral to 6th caudal vertebrae reduced, open pore over sacral area (spina bifida). Agenesis of pollices on left and right front feet, 3rd digits reduced with ageneses of second phalangeal bone and claw. Slight hindlimb malrotation.” This study was re-examined by DPR because of the vastly different interpretation of the study by persons or parties other than the Medical Toxicology Branch reviewer of 1990. Original study investigators, a review by J. C. Killeen Jr. of Ricerca, and the review of U.S. EPA Toxicology Branch of HED all concluded that findings were **not indicative of developmental treatment effects at any dose level. The reported data do not, however, rule out a related cluster of mid-

line malformations. Potentially useful data to further evaluate this study would include historical control data on malformations of Froxfield SPF Rabbits, and identities of males used for pooled sperm in the present study. Availability of validated “estimated no-effect level” (ENEL) procedures allows this study to be re-classified as **acceptable** by DPR. Initially reviewed by Morgan on 12/24/90; re-examined by Aldous on 5/27/08. [NOTE: Study still indicates a “possible adverse effect.”]

NOTE: 51746-0015 090685 is the pilot study using pregnant rabbits associated with above Record No. 090686. Record No. 090687 was the preliminary general toxicity study involving a total of 4 non-pregnant rabbits. The latter study involved two females administered progressively higher doses of fosthiazate, beginning at 1mg/kg and ending with 8 mg/kg. The latter dose was clearly not tolerated: investigators reported “scouring and weight loss, including reduced and fluid gastro-intestinal contents” (p. 12 of that report). Record No. 090685 utilized dose levels of 0, 1, 2.0, 2.5 and 5 mg/kg/day on gestation days 6-19. That study found 5 mg/kg/day to be excessive (5/7 does died or were sacrificed humanely by 8 days of treatment). Also, two does at 2.5 mg/kg/day and one doe at 2.0 mg/kg/day died or were sacrificed moribund on study, which deaths followed significant body weight decrements, and which were attributed to treatment. Two fetuses of one 1 mg/kg/day doe had multiple head malformations. Higher dose levels did not indicate developmental toxicity, and overall this study does not show developmental effects. This study supported a high dose for a definitive study of no more than 2 mg/kg/day. Aldous, 3/28/08.

ORIGINAL ONE-LINER FOR ABOVE STUDY: 51746-015; 90685, 90686, 90687; “IKI-1145: Teratology Study in the Rabbit”; New Zealand White rabbit; Life Science Research Limited, England, LSR report no. 89/ISK118/0694, 12/15/89; IKI-1145 Technical, 93.3% purity, lot# 8603, stability not reported; Vehicle: 0.5% w/v aqueous methylcellulose mucilage; 0 (control), 0.5, 1.0, 1.5 and 2.0 mg/kg/day (by oral gavage) from day 6-19 of gestation; 15 pregnant females/dose; No remarkable maternal effects; **Possible Adverse effects (developmental) indicated:** cleft lip, distance between eyes reduced, cyclopia, no olfactory lobes & continuous cerebral hemispheres, and acrania & spina bifida, incidence of each effect was approximately 1% and the severity of the effects increased with increase in dose; Maternal NOEL = 2.0 mg/kg/day, **Developmental NOEL = NOAEL < 0.5 mg/kg/day (cleft lip); Unacceptable and not upgradable**, no developmental NOEL was established. (Morgan, 12/24/90)

GENE MUTATION

** 51746-017; 90689; “IKI-1145 Technical: Reverse Mutation Test”; Salmonella typhimurium strains TA100, TA1535, TA98, TA1537, Escherichia coli WP2 uvrA; The Institute of Environmental Toxicology, Tokyo, Japan; Report No. IET 89-0054; 11/10/89; IKI-1145 Technical, 93.8% purity; lot# 8603; Vehicle-DMSO (0.1 ml/2.7 ml total); 0 (control), 313, 625, 1250, 2500, and 5000 mg/plate (both non-activated and activated, all bacterial strains); positive controls (non-activated: AF-2, ENNG, 9-AA; activated: 2-AA); metabolic activation system: rat liver S9 fraction induced with Aroclor 1254; 3 plates per concentration, 2 experiments; Test article: no increase in revertant colonies above vehicle control; positive controls: significant increase in revertant colonies in all of the chemicals tested; Study **acceptable**. (Moore, 12/28/90)

**51746-0064 221588 Lloyd, J. M., "IKI-1145 Technical: investigation of mutagenic activity in the TK+/- mouse lymphoma cell mutation system," Life Science Research Limited, Eye, Suffolk, England, 1/20/93. Laboratory Study # 92/ISK192/0841. Fosthiazate (IKI-1145 Technical), purity 95.3%, Batch 7391, was tested in L5178Y TK+/- mouse lymphoma cells. Cell cultures were exposed to test article with or without S-9 for 4 hrs at 2-fold concentration steps of fosthiazate, up to the highest levels indicated by an initial cell viability assay. Cells were then placed in fresh medium and incubated for a 72-hr expression time, with adjustment of cell concentrations to 2×10^5 cells/ml as needed. Selection was then assessed with trifluorothymidine (TFT), after removing aliquots of cells to assess viability. There were two replicates per dose per assay. Positive controls (EMS without S-9 and DMBA with S-9) were functional. There were two assays: the second one adjusted the highest dose levels based on viability patterns observed in the first mutation assay. Both assays were negative with and without S-9. Acceptable, with no adverse effects. Aldous, 5/22/08.

NOTE: 51746-0064 221517 appears to be a redundant record number assignment of Record No. 51746-0064 221588, above.

CHROMOSOME EFFECTS

** 51746-017; 90690; "IKI-1145 Technical: In Vitro Cytogenetics Test"; Chinese hamster lung cell culture (CHL); The Institute of Environmental Toxicology, Tokyo, Japan; Report No. IET 89-0055; 11/10/89; IKI-1145 Technical, 93.8% purity; lot# 8603; Vehicle-DMSO (0.5%); Non-Activated: 0, 0 (vehicle), 12.5, 25, 50, 100, 200 mg/ml; Activated: 0, 0 (vehicle), 46.88, 93.75, 187.5, 375, 750 mg/ml; Positive controls: (non-activated) Mitomycin C (MMC)-0.4 mg/ml, (activated) Benzo(a)pyrene (BaP)-37.84 mg/ml; Activated: metabolic activation system-rat liver S9 fraction induced with Aroclor 1254; 2 independent cultures/concentration; Non-Activated: Cells treated with test article for 24 or 48 hours; Activated: Cells treated with test article for 6 hours, followed by additional culturing for 12 or 18 hours; Frequency of aberrant metaphases: Structural (gaps not included), Non-Activated (all concentrations)-0 (24 hours), 0 to 0.5% (48 hours), positive control-34.5% (24 hours), 59.0% (48 hours); Activated (all concentrations)-0 to 1.0% (12 hours), 0 to 2.0% (18 hours), positive control-39.5% (12 hours), 43.5% (18 hours); Numerical (polyploidy), Non-Activated: 0 (24 hours), 0 to 1.0% (48 hours), positive control-0 (24 hours), 1.5% (48 hours); Activated: 0 to 1.0% (12 hours), 0 to 2.5% (18 hours), positive control-0.5% (12 hours), 0 (18 hours); Study **acceptable**. (Moore, 12/31/90).

**51746-0066 221519 Matsumoto, K., "IKI-1145 Technical: micronucleus test in mice," Institute of Environmental Toxicology, Tokyo, Japan, 10/22/90. Laboratory Study # IET 90-0007. Test article was Fosthiazate (IKI-1145 Technical), purity 93.3%, Lot No. 8603. BDF1 mice were initially tested for toxicity following gavage treatment to fosthiazate (aq. solution, 20 ml/kg b.w.). Test article was uniformly lethal at 100 or 200 mg/kg, with all animals dying by 24 and 19 hours after dosing, respectively. There were no deaths at 50 mg/kg (but rats were sedated for at least one hour at this dose). In Test 1 (time response), 7 mice/sex were orally dosed 24, 48, or 72 hr before sacrifice with 50 mg/kg fosthiazate for bone marrow smear preparation. There were no changes in PCE/NCE ratios nor % micronucleated PCE's at any time period. Positive control (MMC) was highly functional. In Test 2, groups of 6 mice/sex/group were dosed with 12.5, 25, or 50 mg/kg fosthiazate, and sacrificed at 24 hrs for evaluation. Fosthiazate mice were uniformly negative for altered PCE/NCE ratios and % micronucleated PCE's at all dose levels. The positive control, mitomycin C, was functional. Acceptable, with no adverse effects. Aldous, 5/22/08.

DNA DAMAGE

** 51746-017; 90691; “IKI-1145 Technical: DNA Repair Test (Rec-assay)”; *Bacillus subtilis* H17 (rec⁻), M45 (rec⁺); Institute of Environmental Toxicology, Tokyo, Japan; Report no. IET 89-0056; 11/10/89; IKI-1145 Technical, 93.8% purity; lot # 8603; Vehicle-DMSO (20 ml added to disk); 0 (vehicle control), 500, 1000, 2000, 5000, 20000 mg/disk (both non-activated and activated); negative control: Kanamycin-0.1, 0.2 mg/disk; positive controls: Mitomycin C (non-activated)-0.005, 0.1 mg/disk; 2-Amino-anthracene (activated)-5, 20 mg/disk; metabolic activation system: rat liver S9 fraction induced with Aroclor 1254; single plates/concentration; Results: inhibitory zones measured for both H17 and M45 strains were similar for each concentration of test article in both non-activated and activated cultures; results indicate that no cytotoxicity occurred which could be rectified by DNA repair processes; treatment with Kanamycin likewise was similarly cytotoxic to both strains. Positive controls, both non-activated and activated, demonstrated cytotoxic effects which were correctable by DNA repair processes. Study **acceptable**. (Moore, 1/3/91)

NEUROTOXICITY

****51746-0049 221502** Serrone, D. M. and F. Lucas, “An acute neurotoxicity screening study in rats with Technical Fosthiazate (IKI-1145),” Ricerca, Inc. (Painesville, OH) and Pathology Associates International (PAI), Frederick, MD, 03/25/1997. Report No. 5609-95-0034-TX-003. Groups of 10 Sprague-Dawley [CD VAF/Plus®] rats/sex/group were dosed once with 0, 0.4, 10, and either 40 (M) or 20 (F) mg/kg fosthiazate technical (93.8% purity) by gavage in deionized water (10 ml/kg b.w.) in a standard acute neurotoxicity test, including clinical signs observations, FOB, motor activity evaluation, and neurohistopathology. Five/sex/group of these control and high dose groups were perfusion fixed and evaluated for neurohistopathology. An additional 10/sex per group at the above dose levels were allocated for cholinesterase (ChE) evaluations in plasma, RBC’s, and brain. A NOEL for functional or behavioral change is 10 mg/kg, based on remarkably reduced motor activity (such as horizontal and vertical activity, total counts, total distance traveled, and ambulatory time) at the highest dose levels in males (40 mg/kg) and females (20 mg/kg). RBC ChE data did not provide a NOEL: there was significantly reduced RBC ChE activity at Day 0 in males, and a non-significant but plausibly treatment-related reduction in Day 0 females; both at 0.4 mg/kg and above. Brain and plasma ChE values were sharply reduced at 10 mg/kg and above, but were unaffected at 0.4 mg/kg. Brain and RBC ChE inhibition had not resolved within the 2-wk study period, whereas plasma inhibition resolved within 1 wk. Acceptable, **possible adverse effect** (brain ChE inhibition). Aldous, 5/29/08.

51746-0048 221501 Serrone, D. M. and J. Laveglia, “Determination of a no effect level for cholinesterase inhibition in rats with Fosthiazate (IKI-1145 Technical),” Ricerca, Inc., Painesville, OH, 10/20/94. Submitters’ Document No. 5994-94-0096-TX-002. Sprague-Dawley [CD VAF/Plus®] rats were dosed once by gavage with fosthiazate (purity 92.1%, in 10 ml water/kg b.w.) in all tests. Parameters evaluated were inhibition of cholinesterase (ChE) levels in plasma, and of acetylcholinesterase (AChE) RBC’s, and brain (cerebral cortex, cerebellum, and brain stem). Initially, investigators administered a fixed dose of 10 mg/kg fosthiazate to 2 rats/sex, with serial sacrifices at 2, 4, 8, 16, and 24 hr (to find peak response time for plasma and RBC ChE’s only). The range-finding study found marked inhibition of plasma ChE, peaking at

about 3 hr after dosing. RBC inhibition was not evident at this dose level. Sacrifices for the main test were thus performed at 3 hr after dosing. In the main study, 5 rats/sex were dosed at 0, 0.04, 0.4, and 4 mg/kg. Plasma ChE inhibition NOEL = 0.4 mg/kg (based on 36% inhibition in M, and 81% inhibition in F at 4 mg/kg). RBC inhibition was statistically reduced in 4 mg/kg females (a marginal 10% reduction, $p < 0.05$ in a 1-tailed t-test), but not at 0.4 mg/kg. Brain cholinesterases were not inhibited under study conditions. Useful supplementary data. The discussion section of this review concludes that the RBC NOEL is likely to be less than 0.4 mg/kg, based on a later acute neurotoxicity study (Record No. 221502). Aldous, May 8, 2008.

NOTE: ACUTE NOEL'S FOR CHOLINESTERASE INHIBITION: Taken together, study 51746-0049 221502, and supplementary cholinesterase inhibition study, 51746-0048 221501, above, indicate a NOEL for plasma ChE of 0.4 mg/kg, a NOEL for RBC AChE slightly below 0.4 mg/kg, and a NOEL for brain AChE of 4 mg/kg. See Discussion section of review of Record No. 221501 (Aldous, 3/24/08).

****51746-0057 221510** Serrone, D. M. and F. Lucas, "A 90-day neurotoxicity study in rats with Fosthiazate (IKI-1145 Technical)," Ricerca, Inc. (Painesville, OH) and Pathology Associates International (PAI), Frederick, MD, 03/25/1997. Report No. 5610-95-0035-TX-003. Groups of 10 Sprague-Dawley [CD VAF/Plus®] rats/sex/group were dosed in diet to achieve 0, 0.05, 0.5, or 2.5 mg/kg/day fosthiazate technical (93.8% purity) in a standard subchronic neurotoxicity test, including clinical signs observations, FOB, motor activity evaluation, and neurohistopathology. Estimated mean achieved dose levels were 0.07, 0.56, and 2.4 mg/kg/day for males, and 0.08, 0.57, and 2.5 mg/kg/day for females. Five/sex/group of these control and high dose groups were perfusion fixed and evaluated for neurohistopathology. An additional 10/sex/group/interval at the above dose levels were allocated for cholinesterase evaluations in plasma, RBC's, and brain during weeks 5, 9, and 14. Cholinesterase inhibition NOEL = 0.07 mg/kg/day (M) and 0.08 mg/kg/day (F), based on inhibition of plasma and RBC cholinesterase at the mid-dose levels (marked in F and marginal in M). Both sexes showed stronger cholinesterase inhibition at high dose levels, including all brain areas examined: this response being quite marked in females. There were no findings in parameters specific to neurotoxicity studies. The study is acceptable, noting that the motor activity evaluation consisting of a single 15-min block was not ideal. Brain acetylcholinesterase inhibition is a "**possible adverse effect.**" Aldous, 5/29/08.

**** 51746-011; 90681;** Acute Delayed Neurotoxicity; 817; Hen; Life Science Research Limited, Eye, Suffolk, England, Report #89/ISK104/0850, 10/25/89; IKI-1145 Technical; 18 animals; single doses of 20 mg/kg in corn oil on day 1 and on day 23 (in survivors); 44 day observation period; mortalities- 9/18 (7 deaths attributed to cholinergic effects); **Possible adverse effect:** Delayed neurotoxicity (acute delayed onset ataxia); observations- marked cholinergic responses and associated motor impairment including reduced activity, peripheral vasodilation, unsteadiness, drooped wings, resting on hocks, and occasional clonic convulsions clearing shortly after exposure; unsteadiness with reduced activity after being overtly normal for period of several days in 2 animals (days 4 through 8 in one and days 7 through 11 in the other) with the condition of both animals deteriorating- signs of inattention, inability to stand, splayed legs, head on the floor, slightly unsteady gait- to the point that both were killed in extremis, the former on day 13, the latter on day 26; necropsy- minimal focal gliosis of the lumbar-sacral region of animal killed in extremis on day 13; NOEL < 20 mg/kg; **Acceptable.** (Corlett, 2/4/91).

012; 90682; “IKI-1145 Technical: Toxicity Study by Dietary Administration to CD Rats for 13 Weeks Followed by a 10 Week Reversibility Period”; Rat; 821; Life Sciences Research Ltd., Eye, Suffolk, England; LSR Report 870373; 11/16/89; IKI-1145 Technical (Lot No. 8603); Diet: 0, 1.07, 10.7, 53.6, 429 ppm (M: 0, 0.08, 0.77, 4.12, 36.37 mg/kg/day, respectively; F: 0, 0.09, 0.89, 4.74, 41.03 mg/kg/day, respectively); 10 rats/sex/group for 13 weeks, additional 10 rats/sex/group (0, 429) observed for a 10 week recovery period (not dosed); Mortalities: 1 male (0), 1 female (0) during dosing period, 1 male (0) during recovery period; **adverse effects indicated:** tachypnea, emaciation, tremors and nervousness in the 429 ppm group, clearing by week 3-4 of dosing; dose-related inhibition of plasma and erythrocyte cholinesterase activity (10.7, 53.6, 429) ($p < 0.05$; 80-7% of control) week 13, largely recovered by week 4 post-dosing; significant brain cholinesterase inhibition (53.6, 429) ($p < 0.001$; 77-10% of control) week 13, recovered by week 10 post-dosing; adrenal glands: tissue/body weight ratio increased significantly (429), cytoplasmic vacuolation of zona fasciculata, zona glomerulosa dose-related, effects reversible; NOEL (M/F)=1.07 ppm; Acceptable. (Corlett and Moore, 3/18/91).

51746-0052 221505 Broadmeadow, A., “IKI-1145 Technical: preliminary toxicity study in dietary administration to CD rats for four weeks,” LSR, Eye, Suffolk, England, Nov. 6, 1989. Groups of 10 CD rats/sex were fed diets containing 0, 0.5, 1, 5, 10, 100, or 400 ppm fosthiazate (93% purity) in this range-finding study. Findings of greatest potential interest are brain AChE inhibition: (44% and 84% inhibition in 100 ppm and 400 ppm males; 73% and 86% in corresponding females). Statistically significant inhibition was also observed in females at 5 and 10 ppm (9% and 10%, respectively). The latter observations are equivocal, in that the above subchronic (13-wk) study did not find brain AChE inhibition in either sex at 10.7 ppm (p. 69 of Record No. 090682). The lowest detectable circulating ChE inhibition was: (Males) 100 ppm for plasma BuChE and AChE, and 5 ppm for RBC AChE, and (Females) 5 ppm for plasma BuChE and AChE, and 10 ppm for RBC AChE. This study does not appear to warrant a DPR worksheet. See also ChE analyses in a supplementary 18-wk study, below. Aldous, 2/29/08.

51746-0051 221504 (anonymous) “TO-1145: analysis of cholinesterase activity in the rat administered via diet for 18 weeks,” Central Research Institute, ISK, Kusatsu, Shiga, Japan, July 1986. Document No. ISK/258. Fosthiazate (designated as TO-1145), 94.7% purity, was administered to groups of 10 CD rats/sex/group at 0, 0.5, 1, 5, or 10 ppm. Only cholinesterase (ChE) values were assessed. Effects in males were limited to a 19% inhibition of RBC AChE at 10 ppm (NOEL = 5 ppm). Females displayed inhibition of plasma AChE (30% and 55% inhibition at 5 and 10 ppm, respectively) and plasma BuChE (36% and 63% inhibition at 5 and 10 ppm, respectively) (NOEL = 1 ppm). Females also had a 24% inhibition of RBC AChE at 10 ppm (NOEL = 5 ppm for this parameter). There was no inhibition of brain AChE in this dose range. This is a useful non-guideline supplementary study. Aldous, 2/29/08.

****51746-013; 90683;** “IKI-1145 Technical: Toxicity Study by Oral (Capsule) Administration to Beagle Dogs for 13 Weeks;” Dog; 821; IKI-1145 Technical, Lot# 8603; Life Sciences Research Ltd, Suffolk, England; LSR Report# 87/0219; 11/14/89. Key associated submissions which followed also have DPR worksheets, namely (1) 51746-0055 221508 The Pathology Working Group (PWG) peer review of adrenal cortex slides (LSR Report 87/ISK 090/0219), 8/31/95; and (2) 51746-0054 221507 “Statistical analysis of plasma cholinesterase activity in a 90-day dog study with technical fosthiazate,” 12/4/95, Ricerca Document # 6610-95-0249-TX-001. NOTE: This study was upgraded to **acceptable** by Moore (6/11/93 worksheet: no associated new records) because the study provided sufficient information to establish dose levels for chronic toxicity study. The original one-liner for Record # 090683 is updated here to include original

and supplementary information. Dosing: Control (corn oil), 0.054, 0.11, 0.54, 5.4 mg/kg/day in capsules; daily, 13 weeks; 4 animals/sex/group. There were no premature deaths, and no treatment-related clinical signs. Hematology: (F) (5.4)-low packed cell volume (89.1% of control), hemoglobin (89.8%), RBC count (85.8%). Cholinesterase inhibition was observed at the top two dose levels. Plasma butyrylcholinesterase (BuChE) activity was greatly reduced in both sexes after 6 and 12 weeks at 0.54 mg/kg/day (reductions of 41-42% in M, and 57-58% in F), and at 5.4 mg/kg/day (reductions of 61-66% in M, and 61-64% in F). Similarly, plasma acetylcholinesterase (AChE) activity was reduced in both sexes after 6 and 12 weeks at 0.54 mg/kg/day (reductions of 36-37% in M, and 50-54% in F), and at 5.4 mg/kg/day (reductions of 53% in M, and 55-57% in F). At 0.54 mg/kg/day, RBC AChE was reduced in F only (19-26%), whereas at 5.4 mg/kg/day, RBC AChE was reduced markedly in both sexes (reductions of 68-81% in M and 66-79% in F). Brain AChE was affected only at 5.4 mg/kg/day (reductions of 23% and 32% in M and F, respectively). Adrenal glands of 5.4 mg/kg/day males weighed 26% more than controls, a tissue/body weight increase of 29%. NOEL for histopathology of the adrenal cortex = 0.54 mg/kg/day. Key findings were modest but consistent increases in severity of hypertrophy and increased degree of pallor of the zona glomerulosa and zona fasciculata in both sexes. The NOEL for histopathology of the adrenal cortex is different from that proposed by the investigators (0.11 mg/kg/day), and from that of the 1990 DPR reviewer, who noted that adrenal microscopic change appeared to extend to the lowest dose in females. The PWG analysis (consensus evaluation by 5 pathologists blind to treatment group) provided the definitive LOEL of 5.4 mg/kg/day and NOEL of 0.54 mg/kg/day. A **possible adverse effect:** brain AChE inhibition. (Moore, 12/19/90; re-examined with supplementary data by Aldous, 2/21/08).

51746-0055 221508 [Supplemental to: Document # 51476-0013, Record # 090683]. "IKI-1145 Technical: Toxicity study by oral (capsule) administration to beagle dogs for 13 weeks (ISK 090): Pathology Working Group peer review (LSR Report 87/ISK 090/0219)," Experimental Pathology Laboratories, Inc. (pathology reevaluation), 8/31/95. This record has two parts, each concerning Pathology Working Group (PWG) re-evaluations of adrenal tissue slides of dog studies. The part relating to the dog subchronic study comprises pages 18-148. The PWG consisted of 5 pathologists refereed by Dr. Jerry F. Hardisty of Experimental Pathology Laboratories, Inc. (EPL). The PWG conducted a "blind" examination of coded adrenal gland slides from all dogs on study. NOEL for adrenal cortex histopathology = 0.54 mg/kg/day (a change from the 1990 DPR review of the primary report). Key findings were modest but consistent increases in severity of hypertrophy and increased degree of pallor of the zona glomerulosa and zona fasciculata in both sexes. Findings of the PWG are considered as the definitive diagnoses for adrenal histopathology, to be reflected in the revised 1-liner of the primary report of Record # 090683. Aldous, 2/14/08.

51746-0054 221507 Supplemental to 51476-0013 090683 (subchronic dog), "Statistical analysis of plasma cholinesterase activity in a 90-day dog study with technical fosthiazate," Ricerca, Inc., Painesville, OH, 12/4/95. Ricerca Document # 6610-95-0249-TX-001. This report addresses the NOEL for plasma BuChE in females, for which there was significant inhibition observed in Record No. 090683 in 0.11 mg/kg/day females. About 30% of the variability within treatment groups in control females and in the two lower dose groups of females was apparently associated with individual dog baseline differences, which could be factored in analyses using pre-treatment enzyme activity values. When values were adjusted for pre-treatment activities, there were no significant differences in 0.11 mg/kg/day females for BuChE activity. Note that plasma AChE inhibition was significant in the original subchronic study report for 0.11 mg/kg/day females at both sampling times. Plasma AChE were not

presented in this report, but were evaluated by this DPR reviewer for completeness. When adjusted for pre-study activities, plasma AChE inhibition was not significant for 0.11 mg/kg/day females. Aldous, 2/19/08.

51746-0053 221506 See more details in the above "CHRONIC TOXICITY, DOG" section. This report describes the evaluation of dog chronic and subchronic study adrenal gland slides by Dr. D. A. Banas. Later these slides were examined by a refereed Pathology Working Group (Record No. 221508), whose report supercedes earlier evaluations. Aldous, 2/20/08.

51746-0050 221503 Aughton, P. "IKI-1145 Technical: Preliminary Toxicity Study by Dietary Administration to Cd-1 Mice for Four Weeks," Life Science Research Ltd. Eye, Suffolk, England, Nov. 6, 1989. Document No. 86/ISK070/307. Twelve CD-1 mice/sex/group were dosed in diet for 4 wks with 0, 5, 20, 100, or 400 ppm of fosthiazate (92.6% purity) in a standard subacute range-finding study (with no cholinesterase analysis). NOEL = 100 ppm (transient body weight loss in F during days 0-3, increased adrenal absolute and relative weights (F), increased relative liver weight (M), kidney findings of focal tubular basophilia (significantly elevated in both sexes). Useful supplementary data. Aldous, 12/28/07 (no worksheet).

**51746-0056 221509 Broadmeadow, A., "IKI-1145 Technical: Twenty-one day dermal toxicity study in rats," Life Science Research, Ltd. (Eye, Suffolk, England), 11/16/89. Submitter's Document # 89/ISK111/0200. Five CD rats/sex/group were dosed daily with 0, 0.5, 2.5, 25, or 250 mg/kg/day Fosthiazate (IKI-1145 Technical), purity 93.6%, for 6-8 hours daily, for 21 consecutive days. Material was formulated in corn oil, 1 ml/kg b.w., and held under an occlusive dressing. Cholinesterase NOEL's are 2.5 mg/kg/day for males (based on statistically significant inhibition of plasma BuChE and RBC AChE) and 0.5 mg/kg/day for females (based on statistically significant inhibition of plasma BuChE, plasma AChE, and RBC AChE). Brain AChE was significantly inhibited at 25 and 250 mg/kg/day in both sexes, for a NOEL of 2.5 mg/kg/day. All other definitive toxicity was limited to the 250 mg/kg/day group, including decreased survival (deaths or humane sacrifices of 2/5 males and 4/5 females during days 2 to 6), decreased body weight gain, and decreased food consumption. Deaths were often preceded by clinical signs such as "emaciation, torpor, tremor, and hunched posture." The only treatment-related histopathology observed in both terminal survivors and non-survivors was vacuolation of the zona fasciculata of the adrenal cortex (observed in 4 males and 2 females). Decedents occasionally displayed histopathology of the keratinized surfaces of the stomach, such as submucosal edema, acanthosis, and ulceration. Treated skin was apparently unaffected by treatment. Aldous, May 5, 2008.

METABOLISM (includes studies on fate of metabolite, 2-butanefulfonic acid)

51746-0070 to -0077 record series 221523-221531. T. A. Magee was the primary author on all studies in this series. All studies were performed at Ricerca, Inc., Painesville, OH, between 1992 to 1994. These 8 studies on the metabolism of Fosthiazate (IKI-1145 Technical) address current needs for this compound, even though many metabolites are not identified. Generally there are parallel studies for ^{14}C label of the ring carbons, or of carbons on the S-sec-butyl group. All studies employed CrI:CD® BR VAF/Plus® rats. Usually rats were dosed once by gavage (in corn oil vehicle) at 2 or 20 mg/kg. Fosthiazate was efficiently absorbed. Parent compound was not evident in urine (the primary route of excretion). Metabolism was rapid, and both the ring and S-sec-butyl groups were amenable for attack. The ring was typically opened to expose a distal sulfur group, which was subject to methylation and oxidation. These products, leaving most of the parent structure intact except for ring opening and distal sulfur modifications, accounted for only about 7% of administered dose in males, and 18% in females. A small amount of GSH-conjugation products of the S-sec-butyl were obtained. In general, most of the metabolites obtained, regardless of the label placement, showed substantial degradation. Either labeled substituent could be metabolized to release labeled CO_2 (accounting for 7-10% of S-sec-butyl carbon label, compared to 3-4% of ring carbon label). In addition, about 1% of S-sec-butyl carbon was obtained as exhaled volatile organics other than CO_2 . The S-sec-butyl label had a shorter initial plasma disappearance half-life than did the ring-label (about 5 hours, vs. 12-15 hours). Much of the ring-labeled urinary metabolite content was composed of "small, polar, and apparently uncharged components" (42% of administered dose in males and 27% in females). It appears that these included many natural molecules which could be incorporated into tissues. Indeed, 9-11% of administered label following administration of ring-labeled fosthiazate still resided in body tissues after 7 days, compared to 1-2% following S-sec-butyl label. Tissues which concentrated the heaviest labeling at 7 days after ring-labeled fosthiazate were heart, lung, and liver. At least one metabolite identified with ring-labeled treatment, acetamide, constituted 2-3% of recovered label. The review to Record No. 221525 noted that acetamide is carcinogenic in rats at levels much higher than could be obtained from fosthiazate administration. Aldous, 3/18/08 (see reviews of individual records for details).

51746-0068 221521 Ho, M. D., D. B. Johnson, and J. Laveglia, "Pilot study to evaluate the distribution and excretion of ^{14}C -2-butanefulfonic acid in rats after intravenous injection," Ricerca, Inc., Painesville, OH, Feb. 11, 1993. Document No. 5246-92-0096-AM-001. One male SD rat was dosed once with ^{14}C -2-butanefulfonic acid (BSA), [93.3% purity, 10 mg/kg, in 10 ml/kg saline as vehicle]. Investigators evaluated expired air, urine, and feces for 24 hrs, at which time rats were killed with collection of tissues (heart, lungs, kidneys, liver, adrenals, GI tract, and residual carcass). Residues were evaluated by LSC. Urine displayed one peak, determined to be parent BSA based on HPLC retention and MS pattern. Label was recovered almost entirely in urine (urine plus cage wash accounted for over 98% of recovered label). About 1% of recovered dose was obtained in GI tract, and about 0.5% in feces. Less than 0.01% of administered dose was found in other protocol tissues. Only about 0.03% of administered dose was found in expired air. Useful information on fate of this metabolite of fosthiazate, shown to be efficiently absorbed and efficiently excreted with no detectable metabolism. No DPR worksheet. Aldous, 3/6/08.

51746-0069 221522 Liu, Y., M. C. Savides, and J. Laveglia, "Pilot study to evaluate the distribution and excretion of radiolabel following oral administration of ^{14}C -2-butanefulfonic acid to Sprague-Dawley rats," Ricerca, Inc., Painesville, OH, 7/12/94. Document No. 5246-92-

0218-AM-001. Two male SD rats were dosed once by gavage with ^{14}C -2-butanefulfonic acid (BSA), [93.3% purity, 10 mg/kg, in 10 ml/kg water as vehicle]. Investigators evaluated expired air, urine, and feces for 48 hrs, at which time rats were killed with collection of tissues (heart, lungs, kidneys, liver, adrenals, GI tract, and residual carcass). Residues were evaluated by LSC. Urine displayed one peak, determined to be parent BSA based on HPLC retention and MS pattern. Feces were not evaluated for metabolite characterization. Label was recovered mostly in urine (63-89% of administered dose), with much lesser amounts in feces (10-28% of dose) and cage wash (3-6% of dose). Amounts remaining in GI tract or in tissues were minuscule. Only about 0.03% of administered dose was found in expired air. Useful information on fate of this metabolite of fosthiazate, shown to be efficiently absorbed and efficiently excreted with no detectable metabolism. No DPR worksheet. Aldous, 3/6/08.

51746-0070 221523 Magee, T. A., J. P. Marcinişzyn, and D. B. Johnson, "Study to evaluate the distribution and excretion of ^{14}C -(R)-IKI-1145 in rats following repeated dosing," Ricerca, Inc., Painesville, OH, 5/20/93. Document No. 5139-91-0429-AM-001. Groups of 5 CrI:CD® BR VAF/Plus® rats/sex per group were dosed daily for 14 days by gavage (in 2 ml/kg corn oil vehicle) with fosthiazate at 2 mg/kg. Rats were then administered 2 mg/kg labeled fosthiazate, and were killed after 24 hrs or 168 hrs, at which time tissues (including blood) were collected for radiolabel analyses. Urine, feces, and expired air were collected at intervals after dosing. Unlabeled fosthiazate (IKI-1145) was 99% pure, and ring-labeled fosthiazate was >99% pure. There was no apparent effect of pre-treatment on metabolism (compare especially to Record No. 221529). Patterns of excretion and tissue residues over time were comparable to single dose treatment. IKI-1145 is rapidly absorbed, metabolized, and excreted. Urine was the major route of excretion, whereas feces and expired air were minor routes. Neither sex-related differences in route or rate of excretion were observed. Excretion was essentially complete by 24 hours after dosing. At 24 hours, 75 to 82% of administered dose was eliminated. By 168 hours after termination, 70% and 13.2% of administered dose was excreted in urine and feces, respectively. About 5.3% was eliminated as CO_2 . This report is a valid portion of the metabolism study series. Aldous, May 7, 2008.

51746-0071 221524 Magee, T. A., J. P. Marcinişzyn, and J. Laveglia, "Study to measure the pharmacokinetics of ^{14}C -(B)-IKI-1145 in the blood of rats," Ricerca, Inc., Painesville, OH, 5/20/93. Submitter's Document No. 5093-91-0398-AM-001. Groups of 5 CrI:CD® BR VAF/Plus® rats/sex per group were dosed once by gavage (in 2 ml/kg corn oil vehicle) with fosthiazate, either 2 or 20 mg/kg. Unlabeled fosthiazate (IKI-1145) was 99% pure, and S-sec-butyl-labeled fosthiazate was >99% pure. Blood was sampled via orbital sinus at intervals over 7 days. Highest blood concentrations were obtained at about 20 min. Estimated peak blood levels were 3% of administered dose (low dose rats) or 2.2% of dose (high dose rats). At 168 hrs, about 0.18% of administered dose remained in blood regardless of sex or dose. Median initial half-life estimates (defined as peak blood concentration time until 12 hrs) were 4.9 or 5.1 hrs for low dose males and females, respectively. High dose initial half-life estimates were 5.6 and 6.1 hrs for males and females. Median half-life values for the later phase of 18 to 168 hrs were 112 hrs for 2 mg/kg males and females, 96 hrs for 20 mg/kg males, and 85 hrs for 20 mg/kg females. This report is a valid portion of the metabolism study series. Aldous, 3/17/08.

51746-0072 221525 Magee, T. A., Panthani, A. M., and Murray, M. D., "Study to identify the metabolites of ^{14}C (R)-IKI-1145 in the urine of rats," Ricerca, Inc., Painesville, OH, 10/19/94. Document No. 5096-91-0375-AM-001. Seven CrI:CD® BR VAF/Plus® rats/sex were dosed once by gavage (in 2.2 ml/kg corn oil) with 22 mg/kg ring- ^{14}C -fosthiazate prior to assessing

distribution of radiolabel and identifying major metabolites. Label distribution, as percent of administered dose in males and females, respectively, was urine (63.6 and 55.3%), feces (13.5 and 11.2%), expired air (presumed to be CO₂) (3.2 and 4.4%), cage wash (0.7 and 3.5%), and all tissues including carcass (12.9 and 13.1%; 2.0 and 3.5% being g.i. tract and contents), for a total recovery of 93.8 and 87.8% in males and females, respectively. Urinary excretion was high immediately after dosing (43% and 27% of administered dose was recovered within 6 hours of dosing in males and females, respectively): this collection seemed to contain all major labeled metabolites, and was thus selected for metabolite characterization. Nine peaks migrating near to the solvent front formed the largest cluster of radio-labeled components, none of which were characterized. They were considered to be small, polar, and apparently uncharged based on their mobility on a reverse-phase HPLC column and the minimal influence of a basic ion-pairing agent on their mobilities. These constituted 42% of administered dose in males, and 27% of administered dose in females. It appears that ring carbons were substantially assimilated into the carbon pool, considering together the appreciable labeled CO₂ output, substantial label retention in tissues, and the presence of many small MW labeled components in urine. Three metabolites retained the S-butyl substituent and underwent opening of the thiazolidinyl ring. Together these constituted about 7% of administered label in males and 18% in females. The most abundant of these was (RS)-S-sec-butyl O-ethyl N-(2-methylsulfinylethyl) phosphoramidothioate [designated BESxP]. A couple of analogous metabolites had undergone loss of the S-sec-butyl group; O-ethyl S-hydrogen N-2(methylsulfonyl)ethyl phosphoramidothioate [DBSoS] in particular (4.6% of administered dose in males, and 3.0% in females). Acetamide (a rat liver carcinogen at levels about 1000 x larger than levels observed in this study) constituted 3% and 2% of administered dose in males and females, respectively. No other metabolites appeared to be of concern. This report is a valid portion of the metabolism study series. Presence of acetamide as a metabolite is a "possible adverse effect" of apparently minor importance. Aldous, 3/14/08.

51746-0073 221526 Magee, T. A., and J. P. Marciniszyn, "Study to evaluate the distribution and excretion of ¹⁴C(B)-IKI-1145 in rats," Ricerca, Inc., Painesville, OH, Aug. 12, 1992. Submitters' Document No. 3773-90-0486-AM-001. Five CrI:CD® BR VAF/Plus® rats/sex per dose were dosed once by gavage (in 2 ml/kg corn oil vehicle) with fosthiazate, either 2 or 20 mg/kg. Unlabeled fosthiazate (IKI-1145) was 99% pure, and S-sec-butyl-labeled fosthiazate was >99% pure. Excreta and exhaled air were sampled throughout the 7-day post-exposure period. Major tissues were examined at termination for label. Expired air captured in NaOH traps (presumed to be CO₂) constituted 7-10% of administered dose, with no apparent effect of sex or dose level. An additional 1% of administered dose was captured downstream from the CO₂ traps as "volatile organics." Most of the CO₂ and "volatile organics" were collected in the first 12 hrs. Urinary metabolites accounted for 71-73% of administered dose in males, and a comparable 71 to 76% in females. About 70-80% of the urinary label that was collected was acquired within 24 hrs of dosing, with no obvious sex or treatment effect. Feces contained 7-9% of administered dose in all groups. Most of the label in feces was collected by 48 to 72 hours. Less than 0.1% of administered dose remained in the GI tract at 168-hr termination. The percent of administered label in all tissues combined ranged from 1% to 2%, without effect of sex or dose. No one tissue had uniquely high concentrations of label at termination. This report is a valid portion of the metabolism evaluation. Aldous, 5/14/08.

51746-0074 221527 Magee, T. A. and J. P. Marciniszyn, "Study to evaluate the distribution and excretion of ¹⁴C(B)-IKI-1145 in rats following repeated dosing," Ricerca, Inc., Painesville, OH, 8/18/92. Submitters' Document # 3870-91-0092-AM-001. Ten CrI:CD® BR VAF/Plus® rats/sex/group were dosed once daily by gavage (in 2 ml/kg corn oil vehicle) with unlabeled

fosthiazate at 2 mg/kg/day. On Day 15, rats were administered 2 mg/kg S-sec-butyl-labeled fosthiazate. Five/sex were sacrificed at 24 hr after dosing with label; the other 5/sex were sacrificed after 7 days. Study design for the 7-day group was thus parallel to study 51746-0073 221526 (except for the 2-wk pre-treatment in the present study). There was no apparent effect of the pre-treatment on distribution and excretion patterns. Fate of label in the 7-day rats in this study in percent of administered dose was: urine (73 and 74% in M and F, respectively), feces (8 and 9% in M and F), expired air as CO₂ (9 and 8% in M and F), expired air as volatile organics (0.5 and 0.6% in M and F), and all tissues combined (1.8 and 1.2% in M and F). Like Record #221526, no sampled organ had specific radioactivity higher than 2x that of whole blood at day 7 sacrifice. Tissue sampling at 24-hr sacrifice [excluding g.i. tract, due to insufficient time for normal passage of labeled luminal contents] found liver to have the highest specific radioactivity relative to whole blood (liver/blood ratio 4.4 in M and 2.3 in F), followed by lung (lung/blood ratio 2.0 in M and 1.6 in F), with kidney and adrenal specific concentrations slightly lower than lung, and most organs or tissues similar to or lower label concentration than whole blood. Report is a valid portion of the metabolism evaluation. Aldous, 3/17/08.

51746-0075 221529 Magee, T. A., J. P. Marciniszyn, and J. Laveglia, "Study to evaluate the distribution and excretion of ¹⁴C(R)-IKI-1145 in rats," Ricerca, Inc., Painesville, OH, 8/18/92. Document No. No. 3968-91-0198-AM-001. Groups of 5 Crl:CD® BR VAF/Plus® rats/sex per group were dosed once by gavage (in 2 ml/kg corn oil vehicle) with fosthiazate, either 2 or 20 mg/kg. Unlabeled fosthiazate (IKI-1145) was 99% pure, and ring-labeled fosthiazate was >99% pure. Excreta and exhaled air were sampled throughout the 7-day post-exposure period. Major tissues were examined at termination for label. Expired air contained 5-6% of administered dose (in 1 N NaOH traps, presumed to be CO₂), regardless of sex or dose level. Most of this was collected in the first 12 hours. Amounts of volatile organics collected downstream from the CO₂ traps were minuscule. Urinary metabolites accounted for 66-67% of administered dose in males, and 71 to 73% in females. There was no apparent difference between the dose levels. Over one-half of the urinary label was collected within 6 hrs of dosing in all cases. Fecal metabolites constituted 11-12% of administered dose, regardless of sex or dose. Most of this label was collected within 48 hours. Total percentage of administered dose found in all body tissues combined were 11.0 and 9.4% in 2 mg/kg/day and 20 mg/kg/day males, respectively; and 8.2 and 6.8% in respective females, suggesting appreciable retention. Tissue label was widely distributed at 1 week post-treatment. Highest concentrations of label at 2 mg/kg were in liver, lung, and heart. Radioactivity was clearly **not** concentrated in points of entry (g.i. tract) or circulation (blood), nor in the fat. This appreciable tissue retention, coupled with the significant CO₂ production of labeled carbon from metabolism of the ring component, suggests that carbon from the thiazolidinyl ring is substantially assimilated into the body's carbon pool. This report is a valid portion of the metabolism evaluation. Aldous, 5/16/08.

51746-0076 221530 Magee, T. A., Panthani, A. M., and Murray, M. D., "Study to identify the metabolites of ¹⁴C(B)-IKI-1145 in the urine of rats," Ricerca, Inc., Painesville, OH, Oct. 7, 1994. Submitter's Document No. 3869-91-0091-AM-001. Groups of 7 Crl:CD® BR VAF/Plus® rats/sex were dosed once by gavage (in 2.5 ml/kg corn oil vehicle) with fosthiazate at 18 mg/kg. Non-radiolabeled fosthiazate (IKI-1145), which contained ¹³C on 50% of each of the methyl carbons of the butyl group, was 99.3% pure. S-sec-butyl-2-¹⁴C-labeled fosthiazate was 97.7% pure (by MS). Investigators evaluated fate of fosthiazate in tissues and excreta over 48 hours, and characterized major metabolites found in urine. S-sec-butyl-2-¹⁴C-labeled group in the present study produced about the same distribution of label in excreta as had been observed in Record No. 221529, which utilized ring-labeled ¹⁴C. This suggests that both the ring and the

butyl substituents undergo degradation to release appreciable CO₂ (about 10% of administered dose). Identified large MW metabolites (excluding glutathione products) underwent ring opening, often with subsequent methylation of the ring sulfur. Products of oxidation of this ring sulfur, such as sulfonic acids, sulfoxides, and sulfones, constituted about 20% of administered dose. There were several observed residues of hydrolysis of the S-sec-butyl group, similarly displaying oxidation of the sulfur, with or without methylation of the sulfur. Some of the S-sec-butyl group residues underwent glutathione conjugation and were manifest as N-acetyl cysteine products (not further characterized). This report is a valid portion of the metabolism study series. Aldous, 5/27/08.

51746-0077 221531 Magee, T. A., D. B. Johnson, and J. Laveglia, "Study to measure the pharmacokinetics of ¹⁴C-(R)-IKI-1145 in the blood of rats," Ricerca, Inc., Painesville, OH, 5/26/93. Submitter's Document No. 5217-92-0061-AM-001. Groups of 5 Crl:CD® BR VAF/Plus® rats/sex per group were dosed once by gavage (in 2 ml/kg corn oil vehicle) with fosthiazate, either 2 or 20 mg/kg. Unlabeled fosthiazate (IKI-1145) was 99% pure, and ring-labeled fosthiazate was >99% pure. Blood was sampled via orbital sinus at intervals over 7 days. Highest blood concentrations were obtained between 20 min and 1 hr. Estimated peak blood levels were 2.3% to 2.8% of administered dose (no apparent effect of dose or sex). At 168 hrs, about 0.27% to 0.41% of administered dose remained in blood (no apparent effect of sex or dose). Median initial half-life estimates (defined as peak blood concentration time to 12 hrs post-dosing) were 14.9 or 11.5 hrs for low dose males and females, respectively. High dose initial half-life estimates were 8.6 hrs for both males and females. Median half-life values for the later phase of 18 to 168 hrs were 76 and 66 hrs for 2 mg/kg males and females, respectively; and 92 and 87 hrs for 20 mg/kg males and females, respectively. This report is a valid portion of the metabolism study series. Aldous, 5/27/08.

TOXICITY STUDIES ON METABOLITES OF FOSTHIAZATE

- subchronic and subacute studies

51746-0058 221511 Turck, P. A. and J. Laveglia, "A 28-day feeding study in rats with 2-butanefulfonic acid," Ricerca, Inc. (Painesville, OH), 9/29/93. Sponsor's Document #: 5598-93-0020-TX-002. Ten SD rats/sex/group were dosed in diet with 0, 100, 250, 500, or 1000 mg/kg/day 2-butanefulfonic acid (98.5% purity) to assess subacute effects of this metabolite of fosthiazate. There were no treatment effects observed: NOEL = 1000 mg/kg/day for both sexes. Useful supplementary data. Aldous, May 7, 2008.

- mutagenicity studies

51746-0063 221516 Mizens, M. and J. Laveglia "Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test) with a confirmatory assay with 2-butanefulfonic acid," Microbiological Associates, Inc., Rockville, MD, and Ricerca, Inc., Painesville, OH, 10/27/93. Submitter's Document No. 5196-92-0403-TX-003. This study evaluated the fosthiazate metabolite, 2-butanefulfonic acid, in a standard Ames test with and without S-9. There were 2 assays, with 3 reps per dose. Plate concentrations were in steps of 3- to 5-fold, with the highest two concentrations being universally 1000 and 5000 µg/plate. This appears to be an unfortunate wide spread of concentrations, since the range-finding assay had found a sharp dose-response for cytotoxicity in that range. The first mutagenicity assay regularly found some

reductions of the background lawn at 5000 µg/plate in all 5 strains tested (without S-9 only). This change in background lawn was not observed in the second mutagenicity assay. No evident effects on background lawn were observed with S-9 present. This assay found no evidence of mutagenicity with 2-butanefulfonic acid, with or without S-9. Useful supplementary data. Aldous, 3/7/08 (no DPR worksheet).

51746-0065 221518 Mizens, M. and J. Laveglia “L5178Y TK+/- mouse lymphoma mutagenesis assay with a confirmatory assay with 2-butanefulfonic acid,” Microbiological Associates, Inc., Rockville, MD, and Ricerca, Inc., Painesville, OH, 10/27/93. Submitter’s Document No. 5196-92-0404-TX-003. This assay evaluated test cells with and without S-9 at closely-spaced concentration levels up to 5000 µg/ml, in two assays. There appeared to be a slight cytotoxicity at the highest dose tested, so that the dose range evaluated proved to be vigorous and relevant. Positive controls (EMS without S-9 and DMBA with S-9) were functional. There was no increase in mutant frequency with 2-butanefulfonic acid. Valid supplementary study, with no adverse effects. Aldous, 3/7/08 (no DPR worksheet).

51746-0067 221520 Shults, S. K., A. W. Brock, and J. Laveglia, “Micronucleus cytogenetic assay in mice with 2-butanefulfonic acid,” Microbiological Associates, Inc., Rockville, MD, and Ricerca, Inc. (Painesville, OH), 7/15/94. Submitter’s Document No. 5196-92-0405-TX-003. Investigators evaluated 2-butanefulfonic acid (a minor metabolite of Fosthiazate) in a mouse micronucleus assay. Range-finding testing found that the apparent LD_{50} of 2-butanefulfonic acid (purity 98.5%) was slightly under 1500 mg/kg in males, and somewhere between 1500 and 2400 mg/kg in females. As a result, dose levels were 0, 250, 500, and 1000 mg/kg in males and 0, 450, 900, and 1800 mg/kg for females in the micronucleus assays. Five IRC mice/sex/dose/time were tested in the initial study, with sacrifice times of 24, 48, or 72 hrs after dosing (by gavage, in distilled deionized water as vehicle at 10 ml/kg b.w.). There were statistically increased micronucleus counts in 500 and 1000 mg/kg males in the initial assay at the 24-hr pre-treatment interval only. A confirmatory study was conducted in males, with 10 males per dose at the above dose levels for the 24-hr pre-treatment regimen (i.e., twice the numbers as in the original assay for this time interval). The confirmatory study was decidedly negative at all dose levels. Positive control, cyclophosphamide, was functional in both assays. Useful supplementary study, with no adverse effects. Aldous, 3/7/08.

U.S. EPA Data Evaluation Records (DER’s), and Registrant Rebuttals to DER’s
(No DPR reviews or comments of U.S. EPA conclusions unless indicated)

51746-0032 221433 (11/14/02) Fosthiazate Revised Registration Toxicology Disciplinary Chapter (includes presentations of many EPA study summaries, discussion of toxicity endpoints, “classification of carcinogenic potential,” mutagenicity, and remaining data gaps).

51746-0032 221434 Fosthiazate: U.S. EPA HIARC Report of Nov. 12, 2002.

51746-0032 221435 DER - 90 Day Oral in Rats

51746-0032 221436 DER - Subchronic Dog

51746-0032 221437 DER - Teratology in Rabbits

51746-0032 221438 DER - Range Finding Study for Preliminary Teratology Study in Rabbits

51746-0032 221439 DER - Reproduction Study

51746-0032 221440 DER - Toxicity/Oncogenicity Study in Rats

51746-0032 221441 DER - Mutagenicity

51746-0032 221442 DER - Chromosome Aberration

51746-0032 221443 DER - DNA Repair Test

51746-0032 221444 DER - Neurotoxicity in the Hen

51746-0011 091695 DER for rat subchronic study:

51746-0011 091696 DER for initial range-finding study to explore survivability in non-pregnant rabbits, prior to the rabbit developmental toxicity study: 51746-015; 90685, 90686, 90687.

51746-0011 091697 DER for the rabbit developmental toxicity study: 51746-015; 90685, 90686, 90687.

51746-0011 091698 DER for range-finding study (evaluated in pregnant does) for the rabbit developmental toxicity study: 51746-015; 90685, 90686, 90687.

51746-0011 091699 DER for subchronic dog study, 51746-013; 90683.

51746-0011 091700 DER for reproduction study: 51746-016 090688

51746-0011 091701 is the DER for the **interim report** of the rat combined study. See 51746-014 90684, above.

51746-0011 091702 DER for mutagenicity study: 51746-017; 90689 (see above)

51746-0011 091704 DER for chromosomal aberration study 51746-017; 90690 (see above)

51746-0011 091706 DER for DNA repair test 51746-017; 90691 (see above)

REPORTS FROM REGISTRATION LIBRARY PRINTOUT THAT ARE NOT "STUDIES"
RELEVANT FOR REVIEW IN THIS BRANCH

51746-0078 221532 Atropine Sulfate and Pralidoxime Iodide as Possible Antagonists of Fosthiazate (IKI-1145 Technical) in Rats Source: Ricerca, Inc. Painesville, OH, 12/26/1995. This study was mis-classified as a "Study type 822: Repeated Dose Dermal Toxicity/21 Days" study. This study type appears to apply to Worker Health and Safety Branch. Aldous, 3/18/08.

51746-0045 221493 Lucas, F., "Technical Fosthiazate: Summary of Toxicology Data," 4/16/97. This is a toxicology profile for fosthiazate, considering many studies for which DPR has reports. The volume thus discusses NOEL's and LOEL's for a variety of endpoints. These are thus submitters' conclusions, rather reviewable studies. Aldous, 3/20/08.

