

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

TRIAZAMATE

Chemical Code # 5517, Tolerance # 52610
SB 950 # NA

1/24/00

I. DATA GAP STATUS

Combined, rat:	No data gap; Possible adverse effect ¹
Chronic toxicity, dog:	No data gap; Possible adverse effect ¹
Oncogenicity, mouse:	No data gap; No adverse effects
Reproduction, rat:	No data gap; No adverse effects
Teratology, rat:	No data gap; No adverse effects
Teratology, rabbit:	No data gap; No adverse effects
Gene mutation:	Data gap; No adverse effects indicated
Chromosome effects:	No data gap; No adverse effects
DNA damage:	No data gap; No adverse effects
Neurotoxicity:	No data gap; No adverse effects

¹Brain cholinesterase inhibition

Toxicology one-liners are attached.

All record numbers through 170860 were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

indicates a study on file but not yet reviewed.

File name: T178739

P.Leung, 1/24/00

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

**** 053; 170832;** “24-Month Dietary Chronic Toxicity Oncogenicity Study in Rats with RH-7988”; (G.W. Wolfe; Hazleton Washington, Inc., Vienna, VA; Study No. 417-436; 3/31/92); Seventy rats/sex/group were treated in the diet with 0, 10, 250 or 1250 ppm of RH-7988 Technical (purity: 94.9, 95%) for 24 months ((M) 0, 0.45, 11.50, 59.2 mg/kg/day, (F) 0, 0.58, 14.54, 73.70 mg/kg/day). Ten rats/sex/group were euthanized after 12 months on study and examined for histopathological effects. There was no treatment-related effect upon mortality. At various times during the study the mean body weights of the 1250 ppm males were significantly less than those of the controls ($p<0.05$). There was no treatment related effect upon food consumption. Plasma (PChE) and erythrocyte (RChE) cholinesterase activities were significantly less than those of the controls for both the 250 and 1250 ppm treatment groups over the course of the study ($p<0.05$), (PChE: (M) 250 ppm, 42.4 to 69.2% of control, 1250 ppm, 19.2 to 28.6% of control, (F) 250 ppm, 35.0 to 66.3% of control, 1250 ppm, 12.6 to 21.8% of control, RChE: (M) 250 ppm, 71.4 to 82.8% of control, 1250 ppm, 38.2 to 63.2% of control, (F) 250 ppm, 75.4 to 83.7% of control, 1250 ppm, 44.9 to 52.6% of control). Brain cholinesterase activity was significantly lower for the 1250 ppm group ((M) 12 months: 65.9% of control, 24 months: 61.8% of control, (F) 12 months: 64.9%, 24 months: 74.9%) ($p<0.05$). No other significant treatment-related effects were noted for the hematology, clinical chemistry, urinalysis, and ophthalmology. There was no treatment-related effect upon organ weights. The macro- and microscopic examinations did not reveal any treatment-related lesions. **No oncogenic effect indicated. Adverse effect:** brain cholinesterase inhibition; Chronic (M/F): 10 ppm ((M) 0.45 mg/kg/day, (F) 0.58 mg/kg/day) based upon the significant inhibition of erythrocyte cholinesterase in the 250 ppm treatment group); Study **acceptable**. (Moore, 10/27/99)

CHRONIC TOXICITY, DOG

**** 054; 170837;** “RH-7988 (Technical): 52-Week Dietary Toxicity Study in Dogs”; (L. Dickrell; Hazleton Laboratories America, Inc., Madison, WI; Project ID: HLA 6228-104; 12/23/91); Four beagle dogs/sex/group were dosed daily in the diet with 0, 0.1, 0.3, 0.6, 0.9, 15.0, or 150 ppm of RH-7988 (Technical) (purity: 94.9%) for 12 months ((M): 0, 0.0024, 0.0080, 0.017, 0.023, 0.389, 4.16 mg/kg/day, (F): 0, 0.0026, 0.0076, 0.0156, 0.024, 0.392, 4.68 mg/kg/day). There were no treatment-related clinical signs or effects on mean body weights. Mean serum albumin levels were reduced for the 150 ppm females over the course of the study ($p<0.05$). Plasma cholinesterase activity levels were lower for both males and females in the 15.0 and 150 ppm treatment groups over the 52 week period ($p<0.05$). The postprandial activity was less than that of the preprandial samples. Although red blood cell cholinesterase activity was lower for the 150 ppm treatment group, the values were not significantly different from those of the controls. There were no apparent treatment-related effects on other clinical chemical parameters, hematology, urinalysis, or ophthalmology. Brain cholinesterase activity was significantly lower for the females in the 150 ppm group ($p<0.05$). Although the values for the males and

females in the 15.0 ppm group and the males in the 150 ppm group were not significantly different from those of the control animals, the activity levels were 67, 80 and 60% of the control activities, respectively.

There were no treatment-related effects upon the organ weights. Both macroscopic and microscopic examination of the organs and tissues did not reveal any treatment-related effects. **Adverse Effect:** brain cholinesterase inhibition; NOEL (M/F): 0.9 ppm (M: 0.023 mg/kg/day, F: 0.024 mg/kg/day) (based upon dose-related brain cholinesterase inhibition in the 15.0 ppm treatment group); Study **acceptable**. (Moore, 10/25/99)

ONCOGENICITY, MOUSE

** 055, 056; 170836, 170838; "18-Month Dietary Chronic Toxicity/Oncogenicity Study in Mice with RH-7988"; (G.W. Wolfe; Hazleton Laboratories America, Inc., Vienna, VA; Study No. 417-435; 3/10/92); Seventy mice/sex/group were treated in the diet with 0, 1, 50, or 1500 ppm of RH-7988 Technical (purity: 94.9, 95%) for 18 months ((M) 0, 0.13, 6.65, 209.5 mg/kg/day, (F) 0.17, 8.35, 262.4 mg/kg/day). After 55 weeks of treatment, the high dose was lowered to 1000 ppm and maintained at this level to the termination of the study ((M) 127.4 mg/kg/day, (F) 145.9 mg/kg/day). Ten animals/sex/group were euthanized after 12 months of treatment. The high dose group suffered excess mortality with only 26 of 60 males and 30 of 59 females in the group still alive after 15 months of treatment. The predominant clinical observations for these animals were thin with hunched posture, a sign of general ill health. The mean body weight of the high dose animals was lower than that of the controls (between 90 and 92% of the control value). The most significant treatment-related effect was plasma cholinesterase inhibition with the level of activity ranging from 8.4 to 11.2% and 7.2 to 8.2% of the control for the high dose males and females, respectively. Erythrocyte cholinesterase activity for the high dose group was less with the values for the males and females ranging from 68.0 to 84.3 and 58.1 to 78.3% of the control values, respectively. Brain cholinesterase activity was 92.3 and 74.3% and 85.7 and 85.9% for the high dose males and females, respectively after 12 and 18 months of treatment. The only microscopic lesion particularly noted for the high dose animals was inhalation pneumonia. The incidence was most prominent during the first 12 months with 15/36 males and 21/35 females in the high dose group suffering from the lesion. These effects are reflective of the general health status of the animals with no specific etiology for the increased mortality. **No oncogenic effect indicated**. Chronic (Non-neoplastic) NOEL (M/F): 50 ppm ((M) 6.65 mg/kg/day, (F) 8.35 mg/kg/day) (based upon decreased rbc and brain cholinesterase activity in the 1500/1000 ppm treatment group); Study **acceptable**. (Moore, 10/29/99)

REPRODUCTION, RAT

** 059; 170842; "RH-7988: Two-Generation Reproduction Study in Rats"; (H.M. Solomon and W.R. Brown; Rohm and Haas Company, Toxicology Department, Spring House, PA; Report No. 88R-255; 3/31/92); Twenty five rats/sex/group were treated in the diet with 0, 10, 250 or 1500 ppm of RH-7988 Technical (purity: 94.9%) for two generations. The treatment periods included 10 weeks prior to mating, mating, 3 weeks of gestation and 3 weeks of lactation. At that time, 25 F1 animals/sex/group were selected as parents and treated for an additional 10 weeks, followed by mating, and 3 weeks each for gestation and lactation of the F2 generation. No treatment-related mortality resulted from the treatment. Mean body weights and mean food consumption were significantly lower for the 1500 ppm group (P males, F1 males and females) ($p < 0.05$). Plasma and rbc cholinesterase activity was significantly reduced

for the 250 and 1500 ppm treatment groups ($p < 0.01$). None of the reproductive parameters were affected by the treatment. Mean pup weight was less for the offspring in 1500 ppm group (both the F1 and F2 generations). **No adverse effect indicated.** Parental NOEL (M/F): 10 ppm ((M) 0.5 to 1.2 mg/kg/day, (F) 0.6 to 2.0 mg/kg/day) (based upon the significant inhibition rbc cholinesterase activity in the 250 ppm treatment group), Reproductive NOEL: 1500 ppm ((M) 75.4 to 185.1 mg/kg/day, (F) 91.7 to 286.0 mg/kg/day) (highest dose tested), Developmental NOEL: 250 ppm ((M) 12.9 to 30.1 mg/kg/day, (F) 14.5 to 49.2 mg/kg/day) (based upon the reduced mean body weight of the offspring in the 1500 ppm treatment group); Study **acceptable**. (Moore, 10/21/99)

TERATOLOGY, RAT

** 057; 170839; "Oral (Gavage) Developmental Toxicity Study in Rats"; (H.M. Solomon and L.P. Craig; Rohm and Haas Company, Toxicology Department, Spring House, PA; Report No. 87R-117; 5/3/88); Twenty five mated female rats/group were treated orally by gavage with 0, 4, 16, or 64 mg/kg/day of RH-7988 Technical (purity: 95.7%) from gestation day 6 through 15. There were no treatment-related mortalities among the dams. Clinical signs included fasciculations, salivation, rapid breathing and diarrhea which were manifested by the 64 mg/kg treatment group. The mean body weight gain for the high dose females during the treatment period was significantly less ($p < 0.05$) than that of the control animals. Likewise, the mean food consumption of these females was less than that of the control females ($p < 0.05$). There were no treatment-related effects upon fetal development. **No adverse effects indicated.** Maternal NOEL: 16 mg/kg/day (based upon the lower body weight gain, reduced food consumption and clinical signs for the 64 mg/kg/day treatment group); Developmental NOEL: 64 mg/kg/day (no treatment-related developmental effects at HTD); Study **acceptable**. (Moore, 10/18/99)

TERATOLOGY, RABBIT

** 058; 170840; "RH-7988: Oral (Gavage) Developmental Toxicity Study in Rabbits"; (H.M. Solomon and M.F. Lutz; Rohm and Haas Company, Toxicology Department, Spring House, PA; Report No. 89R096; 1/30/92); Twenty one artificially inseminated female rabbits/group were dosed orally by gavage with 0, 0.05, 0.5 or 10 mg/kg/day of RH-7988 Technical (purity: 94.9%) from day 7 through day 19 of gestation. There were no treatment-related mortalities. Clinical signs included diarrhea and/or soiled perinium. These signs were largely limited to the high dose does. The mean body weight gain and food consumption of the 10 mg/kg/day females were significantly less than those values for the control animals ($p < 0.05$). Although the mean male fetal weights for the 0.5 and 10 mg/kg/day litters were significantly less than that of the control animals ($p < 0.05$), there was no apparent dose-related effect. Otherwise, no other possible treatment-related developmental effects were noted. **No adverse effects indicated.** Maternal NOEL: 0.5 mg/kg/day (based upon the lower body weight gain, food consumption and clinical signs noted for the 10 mg/kg/day group); Developmental NOEL: 10 mg/kg/day (No treatment-related developmental effects at HTD); Study **acceptable**. (Moore, 10/19/99)

058; 170841; "RH-7988: Oral (Gavage) Developmental Toxicity Screen in Rabbits"; (H.M. Solomon and M.F. Lutz; Rohm and Haas Company, Toxicology Department, Spring House, PA; Report No. 86R-061; 10/23/87); Eight artificially inseminated female rabbits/group were treated orally by gavage with 0, 1, 10, 30 or 100 mg/kg/day of RH-7988 Technical (purity: 96.9%) from day 7 through

day 19 of gestation. Three of the does in the 30 mg/kg/day group and all of the animals in the 100 mg/kg/day group died. Clinical signs included salivation, lethargy, forward curvature of the spine, fasciculations, and rapid breathing. Some of these signs were observed for all of the treatment groups. There were no apparent treatment-related differences in the 1 and 10 mg/kg/day groups in regard to the number of litters produced and the mean number of live and dead fetuses/litter. There were no treatment-related effects on the fetuses recovered from the does in the 30 mg/kg/day group. **No adverse effects indicated.** Maternal NOEL: <1 mg/kg/day (based upon treatment-related signs in the 1 mg/kg/day treatment group; Developmental NOEL: 30 mg/kg/day (based upon the loss of all of the does in the high dose group); **Supplemental Study.** (Moore, 10/18/99)

GENE MUTATION

52610-060; 170843; “RH-57,988: Salmonella Typhimurium Gene Mutation Assay” (Sames, J. and Frank, J., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Report No. 84R-242, 10/4/84). *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 (all histidine requiring) were exposed to RH-57,988 (Lot No. DPK 0210, 95% pure) at 0 (DMSO), 50, 200, 500, 2000 and 5000 ug/plate, with or without activation by an S9 microsomal fraction, to test for reversion to histidine prototrophy. This concentration range was run once per tester strain, with three replicate plates per concentration. Each concentration was also tested in the presence of histidine, to check for test article-induced bacterial toxicity. Exposure to the test article and bacterial colony formation were for 72 hr at 37°C. Positive controls in the presence of S9 fractions were functional, while those in the absence of S9 were not. The test article caused no increase in the reversion frequency, nor did it cause any bacterial toxicity. **No adverse effects indicated.** Study **unacceptable** (inadequate positive controls without activation). (Vidair, 10/29/99).

52610-060; 170844; “RH-111, 554: Salmonella Typhimurium Gene Mutation Assay” (Sames, J. and Elia, M., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Report No. 92R-0167, 2/2/93). *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 (all histidine requiring) were exposed to RH-111, 554 metabolite (Lot No. NHF2298, 99.3% pure) at 0 (DMSO), 50, 200, 500, 2000 and 5000 ug/plate, with or without activation by an S9 microsomal fraction, to test for reversion back to prototrophy. This concentration range was tested in two independent trials, in 3 replicate plates per test article concentration and 6 replicate plates per control. Exposure to test article and bacterial colony formation were for 72 hr at 37°C. Positive controls were functional. The test article caused no increase in the spontaneous reversion frequency and it was not cytotoxic. **No adverse effects indicated.** Study **supplemental** (Vidair, 10/29/99).

52610-060; 170845; “RH-100,131: Salmonella Typhimurium Gene Mutation Assay” (Sames, J. and Elia, M., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Report No. 92R-0165, 2/2/93). *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 (all histidine requiring) were exposed to RH-100,131 metabolite (Lot No. NHF2303, 98.9% pure) at 0 (DMSO), 50, 200, 500, 2000 and 5000 ug/plate, with or without activation by an S9 microsomal fraction, to test for reversion back to prototrophy. This concentration range was tested in two independent trials, in 3 replicate plates per test article concentration and 6 replicate plates per control. Exposure to test article and bacterial colony formation were for 72 hr at 37°C. Positive controls were functional. The test article caused no increase in the spontaneous reversion frequency and it was not

cytotoxic. **No adverse effects indicated.** Study **supplemental** (Vidair, 11/1/99).

52610-060; 170846; "RH-123,008: Salmonella Typhimurium Gene Mutation Assay" (Sames, J. and Elia, M., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Report No. 93R-0062, 5/6/93). *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 (all histidine requiring) were exposed to RH-123,008 metabolite (Lot No. NHF2387, 95% pure) at 0 (DMSO), 50, 200, 500, 2000 and 5000 ug/plate, with or without activation by an S9 microsomal fraction, to test for reversion back to prototrophy. This concentration range was tested in two independent trials, in 3 replicate plates per test article concentration and 6 replicate plates per control. Exposure to test article and bacterial colony formation were for 72 hr at 37°C. Positive controls were functional. The test article caused no increase in the spontaneous reversion frequency and it was not cytotoxic. **No adverse effects indicated.** Study **supplemental** (Vidair, 11/1/99).

52610-061; 170847; "RH-57,988 Technical CHO/HGPRT Gene Mutation Assay" (Foxall, S., Doolittle, D., and McCarthy, K., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Report No. 86R-073A, 5/17/93). Chinese hamster ovary (CHO) cells were exposed to RH-57,988 Technical (Lot No. LN-1797, 97% pure) for 18 to 20 hr at 37°C without S9 activation (0 to 400 ug/ml giving 103 to 0% cell survival) or 5 hr with S9 activation (0 to 450 ug/ml giving 104 to 21% cell survival). Surviving cells were subcultured for 8 days to allow mutations to be expressed, and then tested for HGPRT locus mutants by resistance to 10 uM 6-thioguanine. Positive controls were functional. In most instances negative control plates yielded no colonies. In the absence of activation, 250 ug/ml of test article gave a maximum of 9.3 resistant colonies per 10⁶ survivors, compared to 0 colonies for the solvent control. It was stated that these values were not significantly different, but no statistical tests were presented. In the presence of 1 mg/ml of an S9 liver fraction, 250 ug/ml of test article gave a maximum of 6.4 resistant colonies per 10⁶ survivors, compared to 1.2 for the solvent control. It was mentioned that this frequency (6.4 per 10⁶) was within the range of historical negative controls. Lowering the S9 concentration to 0.3 mg/ml or raising it to 2.0 mg/ml gave no mutant colonies at the single RH-57,988 concentration tested of 325 ug/ml (24-78% cell survival). **Study unacceptable but possibly upgradeable** with submission of individual plate counts and description of statistical method. (Vidair, 11/1/99).

CHROMOSOME EFFECTS

** 52610-062; 170848; "RH-7988: Test for Chemical Induction of Chromosome Aberration Using Monolayer Cultures of Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation" (Kumaroo, P., SITEK Research Laboratories, Rockville, MD, Project ID No. 0040-3100, 2/26/87). Chinese hamster ovary cells were exposed to test article RH-7988 (Lot No. LN1797, 97% pure) in the absence (nonactivated) or presence (activated) of an S9 microsomal fraction. The nonactivated protocol utilized dose levels ranging from 12.5-300 ug/ml and exposures of 12 or 22 hrs at 37°C, while the activated protocol used 200-475 ug/ml for 2 hr at 37°C followed by an additional 12 or 22 hour incubation. Cytotoxicity was measured by evaluating cell number, mitotic indices and cloning efficiencies. Mitotic cells were processed for metaphase analysis of chromosome aberrations and 50 cells were scored from each of two replicate cultures, at each of two harvest time points. Positive controls were functional. There was no significant increase ($p>0.05$) in the fraction of cells with aberrations or the

average number of aberrations per cell in cells exposed to the test article compared to concurrent negative controls or historical controls, despite induction of measurable cytotoxicity (cloning efficiency was lowered to 76% for the inactivated protocol and to 77% for the activated protocol compared to 100% for both solvent controls). **No adverse effects indicated. Study acceptable** (Vidair, 11/2/99).

** 52610-062; 170849; "Triazamate: Study to Evaluate the Potential of WL 145158 (RH7988) to Induce Micronuclei in the Polychromatic Erythrocytes of CD-1 Mice" (McEnaney, S., Hazleton Microtest Study No. SRS 7/MNT, Hazleton UK Study No. 579/182, 3/19/93). Fifteen mice/sex were injected intraperitoneally on two consecutive days with 10 mg/kg of RH7988 (Batch No. ST92/057, 97.9% pure); a dose which was approximately 65% of the LD₅₀, determined in a range-finding study. Vehicle-only controls (0.5% carboxymethyl cellulose) consisting of 10 mice/sex were injected as above. The positive controls, consisting of 5 mice/sex, received a single intraperitoneal injection of cyclophosphamide at 40 mg/kg. Twenty-four and forty-eight hrs after the second injection (positive control at 24 hr only), five animals/sex/treatment were sacrificed and their marrow from both femurs fixed onto microscope slides for quantification of normochromatic erythrocytes (NCE), polychromatic erythrocytes (PCE), and micronucleated PCEs. At least 2000 PCEs were scored per animal. Treated mice had normal PCE/NCE ratios, indicating that bone marrow toxicity had not occurred. Furthermore, the test article did not induce a significant ($p > 0.05$) increase in the fraction of PCEs with micronuclei, compared to vehicle-only controls. In contrast, cyclophosphamide caused a significant ($p < 0.001$) increase in the fraction of PCEs with micronuclei relative to vehicle-only controls. Results were similar for males and females. **No adverse effects indicated. Study acceptable** (Vidair, 11/3/99).

DNA DAMAGE

** 52610-062; 170850; "RH-57,988 Technical In Vitro Unscheduled DNA Synthesis Assay" (Muller, G., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Report No. 86R-127, 4/30/87). Primary cultures of rat hepatocytes were incubated for 19 hr at 37°C with 10 uCi/ml of ³H-Thymidine and the following concentrations of RH-57,988 Technical (Lot No. LN1797, 97% pure): 0 (1% DMSO), 0.1, 1, 5, 10, 50, 100, 200, 300, 500, 750 and 1000 ug/ml. Three replicate cultures per dose level were processed for autoradiography and measurement of silver grains over the nucleus and cytoplasm. Parallel cultures were used for measurement of cell survival by trypan blue exclusion. Doses of test article ≥ 300 ug/ml lowered survival to $\leq 29\%$ of solvent control and caused test article precipitation. Therefore, only cultures treated with ≤ 200 ug/ml ($\geq 86\%$ survival relative to solvent control) were scored for unscheduled DNA synthesis. The test article caused no increase in the mean net nuclear grain count or in the percentages of cells with net nuclear grain counts of > 6 or > 20 . In contrast, the positive control (2-AAF) induced large increases in all three parameters. **No adverse effects indicated. Study acceptable** (Vidair, 11/4/99).

** 52610-062; 170851; "Triazamate: In Vitro Chromosome Studies with WL145158 Using Cultured Human Lymphocytes" (Brooks, T. and Wiggins, D., Sittingbourne Research Centre, Sittingbourne, England, Project Experiment No. 5602, 2/3/93). Cultures of human lymphocytes were treated with Triazamate (Batch No. F920013, 97.9% pure) at dose levels of 0 (acetone), 78, 156, 312 and 625 ug/ml in the absence of S9 activation, and at 78, 312 and 625 ug/ml in the presence of an S9 activating system. Precipitation occurred above 625 ug/ml. Exposure to the test article was for 3 hr

(activated), 24 hr (nonactivated) or 48 hr (nonactivated). Mitotic cells were sampled for chromosome analysis from duplicate cultures at 24 or 48 hrs after addition of the test article (colcemid added during the final 1.5 hrs to facilitate the accumulation of metaphase cells). Sufficient cytotoxicity was induced by each condition as shown by the following mitotic indices, each relative to the vehicle control: nonactivated 24 hr collection MI = 49% of control at 625 ug/ml; nonactivated 48 hr collection = 54% at 312 ug/ml; activated 24 hr collection = 38% at 625 ug/ml; activated 48 hr collection = 57% at 625 ug/ml. The test article produced no significant increase in the fraction of cells with chromosomal aberrations (excluding gaps) or in the fraction of polyploid/endoreduplicated cells. In contrast, the positive controls significantly ($p < 0.01$) increased the fractions of cells with chromosomal aberrations (excluding gaps). When the fractions of cells with chromosome gaps were measured in the nonactivated system, the test article produced a significant increase ($p < 0.01$) relative to the solvent control at 156 and 312 ug/ml for the 48 hr exposure, but not for the 24 hr exposure. For the activated system, 625 ug/ml caused a significant ($p < 0.05$) increase in gaps in one trial collected at 24 hr, but not in the replicate trial collected at 24 hrs and not at the 48 hr collection. Due to this apparent variability in the cellular response, and the doubtful toxicological significance of this specific measure of chromosome integrity (i.e., gaps), it was concluded that the absence of an effect of the test article on chromosome aberrations (excluding gaps) indicated that the test compound did not damage chromosomes. **No adverse effects indicated.** Study **acceptable** (Vidair, 11/8/99).

NEUROTOXICITY

52610-042, -045 to -047; 170636, 170822 to 170-824; “RH-7988 Technical: Acute Oral (Gavage) Neurotoxicity Study in Rats” (Morrison, R.D. and Gillette, D.M., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Report No. 95R-047, 7/17/96). Ten rats/sex/dose level were administered RH-7988 Technical (Batch No. 0046, 97.8% pure) by single-dose oral gavage at 0 (corn oil), 5, 25 and 75 mg/kg, and monitored over 14 days for signs of neurotoxicity. At study's end the animals were sacrificed and checked for both gross pathological and microscopic lesions. There was no mortality and no effect on 14 day bodyweights. Clinical signs observed on the day of dosing and the following day included: 25 mg/kg fasciculations (1/10 males), yellow-stained anogenital area (1/10 for both males and females), increased incidence of diarrhea (8/10 males); 75 mg/kg fasciculations (5/10 males, 7/10 females), increased incidence of diarrhea (9/10 males, 10/10 females), yellow-stained anogenital area (9/10 males, 10/10 females), red-stained muzzle (1/10 males, 2/10 females). During the first Functional Operational Battery given 0.5-3 hr post-dosing, additional observations of treatment-related behavior were: 25 mg/kg fasciculations (1/10 males in the open arena); 75 mg/kg fasciculations (2/10 males and 3/10 females in the home cage, 5/10* males and 5/10* females in the open arena, * $p < 0.05$), lacrimation (1/10 males, 2/10 females), salivation (1/10 males, 2/10 females). Motor activity was significantly decreased ($p < 0.05$) in high dose females on the day of dosing, both in the total time spent in movement and in the total number of movements. Lastly, both gross and microscopic neuropathology were normal at 14 days. Collectively, these data indicate that the neurotoxicity caused by the test article is reversible. **No adverse effects indicated.** Non-neurological NOEL: (M/F) = 75 mg/kg (based on the absence of non-neurological effects at 75 mg/kg). Neurological

NOEL: (M) = 5 mg/kg (based on an increased incidence of both fasciculations and diarrhea at 25 mg/kg), (F) = 25 mg/kg (based on increased incidence of clinical signs and reduced motor activity at 75 mg/kg). Study **acceptable** (Vidair, 10/19/99).

52610-052; 170830; ‘RH-7988 Technical: Thirteen-Week Dietary Neurotoxicity Study in Rats (Foss, J., Argus Research Laboratories Inc., Horsham, PA, Project ID No. 018-024, Rohm and Haas Report No. 95RC-038, 12/11/96). Twenty five rats/sex/dose level were administered RH-7988 Technical (Lot. No. 0046, 97.8% pure) in their feed for 3 months at 0, 10, 250 and 1500 ppm. Mean daily intake of the test compound was: males 0.6/14.3/86.8 mg/kg, females 0.7/17.1/103.5 mg/kg for 10/250/1500 ppm, respectively. The single mortality was a female from the control group on day 7. Treated animals displayed no abnormal clinical signs. Bodyweights were significantly lower than controls ($p<0.05$) in males fed 1500 ppm (89% of control by final week) and 250 ppm (95% of control by final week). These bodyweight effects correlated with significantly lower food consumption ($p<0.05$) over some weeks for males in the same two treatment groups. In contrast, female bodyweights and food consumption were unaffected. Functional Observational Batteries and Motor Activity tests were performed on weeks -1, 4, 8 and 13. Animals in treated and control groups performed similarly in these tests. Cholinesterase activity was significantly depressed ($p<0.05$) in treated animals of both sexes in all weeks tested (5, 9, 14). Plasma levels ranged from 51-10% of controls for animals in the 250 and 1500 ppm groups, red blood cell levels ranged from 69-22% of controls for the same treatment groups, and brain activity (cortical, cerebellar, hippocampal, hemispheric) ranged from 72-53% of controls for animals at the highest dose level only. There were no gross pathologic changes to the brain at any treatment level (including brain weight), and neurohistology detected very little axonal swelling or nerve fiber degeneration beyond that which was noted in controls. **No adverse effects indicated.** ChE NOEL (M/F) = 10 ppm (based on lower plasma and red blood cell cholinesterase activity in animals fed 250 ppm). Systemic NOEL (M)=10 ppm (0.6 mg/kg/day, based on body weight data) and (F)=1500 ppm (103.5 mg/kg/day, based on no effects at HDT). Study **acceptable** (Vidair, 10/28/99).

METABOLISM

063; 170852; ‘¹⁴C-RH-7988: Pharmacokinetic Study in Rats’; (L.J. DiDonato and S.L. Longacre; Toxicology Department, Rohm and Haas Company, Spring House, PA: Report No. 87R-062; 11/10/88); Male and female rats were dosed orally by gavage or in the diet with ¹⁴C-RH-7988 Technical (specific act.: 13.84 mCi/g, purity not reported). In Groups A, B, C, D, H, and I, 4 males or 4 females/group were dosed by gavage with 0.3 mg/kg of the test material. In Groups H and I, the animals received 3 ppm of the unlabeled test material in the diet for 14 days prior to being dosed with ¹⁴C-RH-7988 on the 15th day. In Groups E, F and G, 4 males or 4 females were dosed by gavage with 30 mg/kg of the test material. In Groups J and K, 3 males or 3 females/time point were dosed by gavage with 30 mg/kg of test material and serially euthanized at 15 minutes, 6 hours and 1 day. In Groups L and M, 4 males/group received 300 ppm of the test material in the diet for 24 hours. In Groups N and O, 4 males/group received 3000 ppm of the test material in the diet for 24 hours. For Groups A, C, and F, whole blood and plasma were collected at 5 and 15 minutes, 1, 3 and 6 hours and 1, 2, and 3 days after dosing. For Groups L and N, whole blood and plasma samples were collected at 15 minutes, 1, 3 and 12 hours and 1, 2, 3 and 4 days after dosing. For Groups B, D, E, G, H, and I, urine and feces were collected prior to dosing and at 6 hours and 1, 2 and 3 days after dosing. For Groups M and O, urine

and feces were collected prior to dosing and at 1, 2, 3 and 4 days after dosing. For Groups B, D, E, G, H and I, animals were euthanized 3 days after dosing and the presence of residual radiolabeling determined in selected tissues. For Groups J and K, the presence of radiolabeling in selected tissues was determined at 15 minutes, 6 hours and 1 day after dosing. The radiolabel was largely absorbed with 92 to 112% of the label recovered in the urine and the tissues. The quantity of dose administered or predosing with unlabeled test material apparently did not affect the absorption. However, closer evaluation of the excretion profile indicated that the 30 mg/kg treatment groups excreted a greater percentage of the radiolabel in the feces (20 to 32% of the administered dose) than did the 0.3 mg/kg treatment groups (10 to 11%). Administration of the test material in the diet resulted in a lower percentage of the test material apparently being absorbed (67.4 and 73.5%) and a greater percentage being excreted in the feces (33 and 25%, respectively). Greater absorption may have occurred. However, no evaluation of the bile was performed. The peak level of radiolabel was apparently 15 minutes after dosing, indicating a rapid distribution throughout the body. The greatest concentrations of radiolabel were in the liver and kidney. The lung, heart, adrenal and thyroid also had elevated levels of the radioactivity. The residual radioactivity in the tissues was minimal 72 hours after dosing. Peak whole blood and plasma levels were achieved 5 to 15 minutes after dosing for the animals receiving the test material by gavage. For the animals receiving the test material in the diet, the peak levels were achieved 12 to 24 hours after provision of the dietary preparations. Three half-lives for the radiolabel in the plasma were determined with the very rapid phase T_{1/2} ranging from 0.3 to 0.6 hours. The rapid phase T_{1/2} ranged from 2.6 to 10.4 hours and T_{1/2} for the terminal phase ranged from 36.5 to 172.9 hours. Study **unacceptable, possibly upgradeable** with the submission of metabolism study data derived from 86R-205 (DiDonato and Hazleton, 1987). (Moore, 11/3/99)

52610-064; 170853; "A Material Balance and Metabolism Study of ¹⁴C-RH-7988 in Rats"; (D. Hamp; Rohm and Haas Company, Toxicology Department, Spring House, PA; Report No. 34-89-31; 6/27/89); Eight rats/sex were dosed by oral gavage with 30 mg/kg of ¹⁴C RH-7988 (spec. act. 13.84 mCi/g, radiopurity: 97.8%) in corn oil. The radiolabel was on the 5-position of the triazole ring. Urine and feces were collected at 6, 24, 48 and 96 hours after dosing. For the males and females, 101.8% and 83.3% of the administered dose was recovered, respectively. The radiolabeled material was predominantly recovered in the first 24 hours (97.4% and 77.0% of the administered dose for the males and females, respectively). The urine and urinary funnel wash were the primary sites of recovery with 77.2% and 63.8% of the administered dose recovered for the males and females, respectively. Among the metabolites recovered, RH-7280 was the predominant one in both the urine and the feces. The amide bond linking the carbonyl carbon to the triazole ring in the 1-position had been hydrolyzed and the carboxy ester bond had been deethylated on the thio linkage to the 5-position of the triazole ring. The other metabolite recovered in a significant percentage in the urine (hydroxy-RH-2855) had been deethylated at the carboxy ester bond and one methyl group on the N,N-dimethyl group had been removed with the second one hydroxylated. None of the parent compound was recovered. **Supplemental** study. (Moore, 11/18/99)

52610-065; 170854; "14C-WL145158 (triazamate, RH7988): Fate of WL145158 in the Rat Following a Single Oral Dose (30 mg/kg)-Supplementary Study"; (K.A. Richardson; Sittingbourne Research Centre, Sittingbourne, Kent, England; Report No. SBGR.93.119; 10/13/93); Twelve male rats were dosed orally by gavage with 30 mg/kg of [triazole-¹⁴C] WL145158 (spec. act. 19.1 µCi/mg;

radiochemical purity: >98%). Urine samples were collected at 6, 24 and 48 hours after dosing. Although feces samples were collected, no analysis of the samples was performed. Urine was the primary route of excretion with 82.6% of the administered dose recovered by 48 hours via that route. The proposed metabolic pathway was 1) the deethylation of the carbonyl ester (RH-0422, 5.1%), 2) demethylation of the N, N-dimethyl group (RH-2855, 10.1%), 3) hydroxylation of the remaining N-methyl group (RH-2855 (OH), 14.8%), 4) removal of the hydroxymethyl group from the nitrogen (RH-16892, 1.5%), 5) hydrolysis of the amide linkage on the 1-position of the triazole ring (RH-7280, 44.9%) and 6) oxidation of the sulfur group to the sulfoxide (RH-0131, 1.3%). **Supplemental** study. (Moore, 11/18/99)

52610-066; 170855; “¹⁴C-WL145158 (RH-7988): Absorption, Distribution, Metabolism and Excretion in Male Dogs following a Single Oral Dose and Comparison of Rat and Dog Metabolite Profiles”; (S.A. Cameron et. al. (dosing protocol), A.J. Mercer and A. Croucher (metabolite identification); Inveresk Research International, Tranent, Scotland and Sittingbourne Research Centre, Sittingbourne, Kent, England; Report No. SBGR.93.194; 12/6/93); Three male dogs were dosed orally by gavage with a target dose of 5 mg/kg of ¹⁴C-WL145158 (RH-7988) (spec. act. 51.31 μCi/mg, radiochemical purity: >99%) and urine, feces and cage wash were collected up to 52 hours after dosing. A total of 95% of the administered dose was recovered by the time of sacrifice with only 0.63% in the carcass or gastrointestinal tract. The urine was the primary site of collection with 65.3% recovered after 52 hours. In addition, 15.9% of the administered dose was recovered in the cage wash and 13.3% in the feces. The liver was the predominant organ in which radiolabel was recovered. However, that represented a minimal amount of the administered dose. The two primary metabolites were formed by ethyl ester hydrolysis (RH-0422) followed by de-carbamoylation (RH-7280). Although the recovered percentages of the metabolites from the dogs were varied from those of the rat, the metabolic pathway demonstrated in the dog was similar to that of the rat. **Supplemental** study. (Moore, 11/19/99)

SUBCHRONIC

52610-049; 170827; “RH-7988: Three-Month Dietary Toxicity Study in Rats” (Bernacki, H., Kulwich, B. and Hazelton, G., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Report No. 87R-112, 6/1/89). Sixteen rats/sex/dose level were administered RH-7988 (Lot No. WZ-28-16, 96% purity) in their feed for 3 months at 0, 50, 500, 1500 and 3000 ppm. Six of the rats/sex/dose level were used primarily for measurement of cholinesterase activity at 3 months. Average daily intake of the test compound was: males 3/31.6/93.8/190.5, females 4/39/117.8/250 mg/kg for 50/500/1500/3000 ppm. By study’s end, mean bodyweights of males fed 1500 and 3000 ppm were 90% and 76% of controls, respectively (p<0.05), while females were 87% and 88% of controls (p<0.05). These effects on bodyweights corresponded to significantly lower food consumption (p<0.05) for a number of weeks at both 1500 and 3000 ppm in both sexes. The few statistically significant (p<0.05) alterations in parameters of clinical chemistry (decreased globulin, total protein, glutamic-oxalacetic transaminase activity; increased inorganic phosphorus) were either considered not to have been treatment-related, or were thought to be of questionable toxicologic significance (decreased glutamic-oxalacetic transaminase activity). Urinalysis detected a significant (p<0.05) acidification of the urine in males fed 3000 ppm (pH 7.95 in controls to 7.25 in treated) and females fed 3000 ppm (8.10 in controls to 7.40 in treated). It was suggested that these decreases in pH were not due to kidney damage, since there was no corresponding gross or histopathologic evidence, but rather that

excretion of the test compound and/or its metabolites was the cause. Mean cholinesterase activity was significantly ($p < 0.05$) inhibited in the plasma of both males and females in a dose-dependent manner at 500, 1500 and 3000 ppm. Red blood cell cholinesterase activity was lower in females at 500, 1500 and 3000 ppm ($p < 0.05$), while in males it was lower at 1500 and 3000 ppm. Brain cholinesterase activity exhibited a dose-dependent drop ($p < 0.05$) in both sexes at 1500 and 3000 ppm. Gross pathology and histopathology did not detect any test compound-induced effects. Statistically significant ($p < 0.05$) alterations in various organ weights (both absolute and relative) were observed at 3000 ppm for males and 500, 1500 and 3000 ppm for females; however, these effects were sporadic and were ascribed to changes in bodyweight. No adverse effects indicated. ChE NOEL (M/F) = 50 ppm (M: 3.22 mg/kg/day, F: 3.87 mg/kg/day; based on decreased cholinesterase activity in the plasma of males and females, and in the red blood cells of females, fed 500 ppm). Systemic NOEL = 500 ppm (decreased body weights). Study acceptable (Vidair, 10/25/99).

52610-048; 170825; "RH-7988: Three-Month Dietary Toxicity Study in Mice" (Quinn, D.L. and Hazelton, G.A., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Report No. 87R-102, 6/1/89). Twenty mice/sex/dose level were administered test article RH-7988 (Lot No. WZ-2816, 96% purity) in their feed for 3 months at 0, 0.5, 2, 25, 250 and 1000 ppm. Ten males and ten females from each dose level were used specifically for measurement of plasma, red blood cell and brain cholinesterase activities at 3 months. Mean daily intake of the test compound for all animals was: males 0.086/.354/4.379/45.768/164.139, females 0.136/.528/6.076/66.728/221.985 mg/kg for 0.5/2/25/250/1000 ppm. There was no treatment-related mortality. Weekly mean bodyweights and mean food consumption were largely unaffected by treatment. Similarly, hematology, clinical chemistry, urinalysis, gross pathology (including organ weights, absolute and relative) and histopathology, all measured at 3 months, were unaffected by the test compound. In contrast, plasma cholinesterase activities were significantly ($p < .05$) lower than control activities at 25, 250 and 1000 ppm for both males (1000 ppm = 11% of control) and females (1000 ppm = 13% of control). In both cases a dose-response was indicated. Additionally, brain cholinesterase activity was 81% of control ($p < .05$) for males at 1000 ppm. No adverse effects indicated. ChE NOEL (M/F) = 2.0 ppm (based on lower plasma cholinesterase activity at ≥ 25 ppm). Systemic NOEL = 1000 ppm. Study not acceptable due to lack of ophthalmology. (Vidair, 10/22/99).

52610-051; 170829; "RH-7988: Two-Week Dietary Range-Finding Study in Male Dogs" (Goldman, P. and Poorman, K., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Study No. 86R-154, 5/31/89). Four male Beagle dogs/dose level were administered RH-7988 (Lot No. LN2052, 99% pure) in their feed for 2 weeks at 0, 140 and 700 ppm. Treatment groups fed 3500 and 7000 ppm were discontinued after 1 week or 1 day of feeding, respectively, due to the severity of clinical signs. The dogs from the 3500 ppm group were fed untreated food for 1 week, followed by sampling for clinical studies. The dogs from the 7000 ppm group were given untreated food for 6 days, followed by 2 weeks of feeding at 300 ppm. No mortality resulted. The most frequent clinical signs were vomiting and diarrhea at dose levels of 140 ppm and above. Bodyweights were reduced at all dose levels, with the greatest effect seen in the 700 ppm group (approximately 75% of controls by 14 days). Food consumption was also lower than controls at 300 ppm and above. In agreement with this latter finding, the rates of daily test article intake during week 1 were [5.07/9.30/10.55/8.75/31.66* mg/kg for 140/300/700/3500/7000 ppm (* = based on a single day

of dosing)] and week 2 [5.24/9.97/11.95 mg/kg for 140/300/700 ppm]. Hematology measurements on day 14 detected decreased eosinophils, decreased lymphocytes and increased segmented neutrophils, all for the 700 ppm dose group. Serum chemistry on day 14 detected decreased alkaline phosphatase (300, 700, 3500 ppm), increased urea nitrogen (700 ppm), increased creatine (300, 700 ppm), decreased inorganic phosphorous (300, 700 ppm), decreased glutamic-pyruvic transaminase (300, 700 ppm), increased triglycerides (700 ppm) and decreased plasma cholinesterase (140, 300, 700 ppm). Two of four animals in the 700 ppm dose group were noted as “whole body thin” at necropsy. When organs were weighed, the adrenals were higher in the 700 ppm animals, both in absolute and relative terms. NOEL not established. Study supplemental (Vidair, 10/27/99).

52610-050; 170828; “RH-7988: Three-Month Dietary Toxicity Study in Dogs” (Dickie, B., Hazleton Laboratories American, Inc., Madison, WI, Project ID No. HLA 6228-10, 7/25/89). Four Beagle dogs/sex/dose level were administered RH-7988 (Lot No. WJZ-28-40, 95.3% purity) in their feed for 3 months at 0, 1, 10, 100 and 400 ppm. Mean daily intake of the test compound was: males .03/.31/3.09/10.75, females .03/.30/3.13/11.21 mg/kg corresponding to 1/10/100/400 ppm. Bodyweights were significantly lower ($p<0.05$) than controls in the 400 ppm group of both males and females, while food consumption was only lower ($p<0.05$) in 400 ppm males. One male in the 400 ppm group died during week 9 and one in the same group was sacrificed moribund in week 12, both with pathological findings suggesting that the animals suffered from malnutrition. Consistent with this suggestion were the clinical signs exhibited by animals in the 400 ppm group including vomiting food, bloating, thin appearance and hypothermia. Increased vomiting of food was the primary clinical sign observed in the 100 ppm group. Weekly physical examinations detected ocular discharge, bloating, thin appearance, hypothermia, slow pupillary response, rectal bleeding, pale mucus membranes, dehydration and emaciation in 7/8 animals fed 400 ppm. At 100 ppm, 5/8 animals exhibited bloating, thin appearance, slow pupillary response and ocular discharge. Heart rates were measurably lower than controls ($p<0.05$) at 400 ppm in males (week 4) and females (weeks 2-4). Slow pupillary response was also observed in 1/4 females at 10 ppm at week 8. The most frequently observed change in clinical chemistry was a drop in total protein ($p<0.05$) relative to controls for males at 400 ppm (weeks 4 and 13) and for females at 100 and 400 ppm (week 4). Albumin decreased ($p<0.05$) and sodium increased ($p<0.05$) relative to controls in males fed 400 ppm at both weeks tested, 4 and 13. Other changes were single occurrences. Plasma cholinesterase activity, measured at 1-3 hr after feeding, decreased ($p<0.05$) in a dose-dependent manner in both males (10, 100, 400 ppm) and females (1, 10, 100, 400 ppm). Red blood cell and brain cholinesterase levels were not affected. Eye pathology was normal, notwithstanding the findings of slight retinal thinning in 1 female at 100 ppm and 2 females at 400 ppm during the ophthalmologic exams at week 13. No adverse effects indicated. ChE NOEL (M) = 1 ppm (based on lower plasma cholinesterase activity in males fed 10 ppm). A no-observed-effect level for plasma ChE was not identified in females. Systemic NOEL=10 ppm (clinical signs). Study **acceptable** (Vidair, 10/27/99).

(Dermal)

52610-043; 170637; “RH-7988 Technical and RH-7988 50WP: Twenty-One Day Dermal Toxicity Study in Rats” (Craig, L., Parno, J. and Gillette, D., Rohm and Haas Company, Toxicology Department, 727 Norristown Road, Spring House, PA, Report No. 95R-048, 8/27/96). Six rats/sex/dose level were treated over 21 days with 15 dermal applications of RH-7988 Technical (Lot

No. 0046, 97.8% purity) or RH-7988 50WP (Lot No. PGF-XI-9, 52% A.I.) at 10, 100 and 1000 mg/kg for the former and 10 mg/kg for the latter. Each application was for 6 hr, followed by washing away of the test article. There was no treatment-related mortality and no effects on mean bodyweights or mean food consumption. Absolute and relative organ weights (kidney, testes, liver) were also normal. Upon necropsy, no treatment-related effects were observed on either gross pathology or histopathology. Blood taken at study's end and analyzed along with brain tissue showed a significant ($p < .05$) inhibition of cholinesterase activity at 100 and 1000 mg/kg relative to controls in red blood cells (max. 67% inhibition), plasma (max. 81% inhibition) and brain (max. 58% inhibition). Other significant ($p < .05$) changes in parameters of clinical chemistry (albumin, total protein) and hematology (white blood cells) in females were not considered to have been treatment-related. **No adverse effects indicated.** NOEL for Systemic Toxicity (RH-7988 Technical): (M/F) = 10 mg/kg (based on inhibition of plasma, red blood cell, and brain cholinesterase activities at 100 mg/kg; (RH-7988 50WP): produced no effects at 10 mg/kg. Dermal NOEL (M/F)=1000 mg/kg/day (no effects at HDT). **Acceptable** (Vidair, 10/21/99).

SUPPLEMENTAL STUDIES

52610-070; 170862; "Range-finding Study with RH-7988 in Dogs"; (B.C. Dickie; Hazleton Laboratories America, Inc., Madison, WI; Project ID. HLA 6228-103; 7/14/89); The study was performed in 8 separate phases. In each of these phases 2 beagle dogs/sex were dosed with the test material. The test material in the first 6 phases was RH-7988 Technical (purity: 94.9%). In Phase I, bleed 1 and 2, the dogs received 100 ppm of the test material in the diet. In bleed 1, the females were refed the test material 6 hours after the initial treatment. In bleed 2, the males were refed 6 hours after the initial treatment. In bleed 1, maximal plasma cholinesterase (ChE) inhibition was noted at 2 hours after dosing for the males (19.6% of 0 time) and at 10 hours (4 hours after redosing) for the females (20.3% of 0 time). Bleed 1 was repeated with plasma ChE being inhibited maximally in the males at 8 hours post-dose (18.5% of 0 time) and at 12 hours (6 hours after redosing) (19.4% of 0 time) for the females. Likewise, in bleed 2, maximal plasma ChE inhibition was noted at 12 hours (6 hours after redosing) for the males (24.9% of 0 time) and at 8 hours for the females (24.6% of 0 time). There was no apparent affect upon rbc ChE activity in either bleed. In Phase I, bleed 3, treatment by oral gavage with doses ranging from 0.4 to 0.7 mg/kg resulted in maximal inhibition of plasma ChE at 1 hour post-dose, males-15.0% of 0 time, females-18.0% of 0 time. There was no treatment-related effect upon rbc ChE activity. In Phase II at the highest dose tested, 1.5 ppm in the diet, plasma ChE was maximally affected at 4 hours post-dose (85% of 0 time value) for both sexes. Rbc ChE activity was not affected. In Phase III, the dogs were treated by oral gavage with doses ranging from 0.003 to 0.1 mg/kg of the test material. Plasma ChE activity was approximately 80 to 85% of 0 time levels at 0.03 mg/kg for both sexes and 40 to 50% of 0 time values for the females and males, respectively at the highest dose. Maximal inhibition was noted at 1 hour after dosing. Rbc ChE activity was not affected by the treatment. In Phase IV, the dogs were treated with 10 ppm of the test material in the diet. Plasma ChE activity was maximally reduced at 2 to 4 hours after dosing with the activity level approximately 50% of the 0 time value for both sexes. The rbc ChE activity level was not affected. In Phase V, the dogs received 1.5 ppm of the test material in the diet. Plasma ChE levels were never less than 83.5% of the 0 time values with maximal effect noted at 10 to 12 hours after dosing. There was no treatment-related effect upon the rbc ChE activity levels. In Phase VI, the dogs were dermally exposed to doses ranging from 0.004 to 0.4 mg/kg of the test material. At 0.4 mg/kg, plasma ChE activity was reduced to 72.2% and 72.5% of 0 time

activity for the males and females, respectively at 24 and 12 hours post-dose, respectively. Rbc ChE activity was maximally affected at 1 hour for the males (90% of the 0 time value) and at 4 hours for the females (81% of the 0 time value). In Phase VII, two dogs/sex were exposed dermally to doses ranging from 0.004 to 4 mg/kg of RH-7988 4EC (a.i.: 47%). At a dose of 0.4 mg/kg, plasma ChE activity was maximally affected at 12 hours after dosing for the males (74.4% of the 0 time value) and at 24 hours post-dose for the females (73.9% of the 0 time value). At 4.0 mg/kg, the plasma ChE activities were at the lowest level 72 hours after dosing (45.3% and 23.2% of 0 time values for males and females, respectively). There was no apparent treatment-related affect upon the rbc ChE activity. In Phase VIII, two dogs/sex were treated dermally with doses ranging from 0.04 to 4.0 mg/kg of RH-7988 25WP (a.i.: 25%). Plasma ChE activity was maximally reduced at 24 hours post-dose at the 0.4 mg/kg dose level (77.2 and 74.9% of the 0 time values for the males and females respectively). At the highest dose, maximal reduction of 64.2% of 0 time value was noted for the males at 48 hours post-dose and 63.5% of 0 time value for the females at 24 hours. Overall, plasma ChE activity levels were affected by the three methods of dosing examined in the study, oral by gavage, oral in the diet and dermal. In the diet, the activity was affected in a dose-related manner with the activity at 85, 50 and 20% of 0 time levels for treatments of 1.5, 10 and 100 ppm, respectively. Likewise, oral gavage treatments resulted in activity levels of 85, 35 to 50 and 15 to 20% of 0 time values for dosages of 0.03, 0.1 and 0.4 to 0.7 mg/kg. Dermal application at 0.4 mg/kg resulted in an activity level of 72% of the 0 time value. Rbc ChE activity was minimally affected using the same dosing regimens. Dermal treatment with the two formulated products resulted in a dose-related reduction in plasma ChE activity. No affect on rbc ChE activity was noted. **Supplemental** study. (Moore, 11/17/99)

52610-070; 170861; "RH-57,988 Technical: Acute Inhalation Toxicity Assessment Study in Rats"; (J.V. Hagan et. al.; Rohm and Haas Company, Toxicology Department, Spring House, PA; Report No. 88R-111; 5/31/89); In Group 1, 55 female rats were exposed to 57 mg/m³ (analytical determination) of RH-57,988 (purity: 94.9%) nose-only for time periods ranging from 0 to 6 hours. The mean MMAD (GSD) value was 1.95 (1.85) µm. Five animals/time point were euthanized at 0, 30, 60, 120, 240 and 360 minutes of the exposure and at 1, 2, 4, 24 and 48 hours after the exposure and the plasma and red blood cell cholinesterase (ChE) activity levels determined. In Groups 2 and 3, 25 female rats each were exposed to 13 and 7 mg/m³ (analytical determination), respectively, of the test material for time periods ranging from 0 to 6 hours. The mean MMAD (GSD) values were 3.65 (2.58) and 2.44 (2.48) µm, respectively. Five animals/time point were euthanized at 0, 240 and 360 minutes of the exposure and at 4 and 24 hours after the exposure. The mean plasma cholinesterase activity was significantly lower for the high exposure group from the first hour of exposure through 2 hours after the exposure (maximal effect: 21% of 0 time value at the end of the exposure) (p<0.05). Likewise, for the 7 and 13 mg/m³ exposure groups, maximal effect was noted at the termination of the exposure (80.1 and 42.4% of the 0 time values, respectively). The red blood cell ChE activity was affected in the high exposure group with maximal effect noted at the end of the exposure (77.9% of the 0 time value) (p<0.05). **Supplemental** study. (Moore, 11/8/99)

52610-070; 170860; "Cholinesterase Inhibition after Oral Administration to Rats"; (R.D. Morrison et. al.; Rohm and Haas Company, Toxicology Department, Spring House, PA; Report No. 88R-157; 5/30/89); In Phase 1, five and forty five female rats were dosed orally by gavage with 0 or 10 mg/kg of RH-57,988 Technical (purity: 94.9%), respectively. Five treated animals/time point were

ethanized at 0.25, 0.5, 1, 2, 4, 6, 8, 24, and 48 hours after dosing and the plasma and red blood cell cholinesterase (ChE) activities were determined. The greatest level of plasma ChE inhibition was achieved at 0.5 hours after dosing (20.3% of control). This level of inhibition was maintained up to 2 hours after dosing. No red blood cell ChE inhibition was demonstrable. In Phases 2 and 3, 5 females or 5 males/time point were dosed orally by gavage with 0, 0.3, 1.0, 3.0, 10.0 mg/kg of RH-57,988 Technical and euthanized 0.5 or 1.0 hours after dosing. In Phase 2, plasma cholinesterase activity was 66.0 and 54.9% of the control activity for the 3.0 mg/kg treatment group at 0.5 and 1.0 hours after dosing, respectively. The effect upon the rbc ChE activity of the females was less remarkable. Maximal inhibition was achieved for the 10 mg/kg treatment group at 0.5 hours after dosing with activity 58.1% of the control value. In Phase 3, any treatment-related effect on plasma ChE activity was limited to the 3.0 and 10.0 mg/kg treatment groups at 0.5 hours after dosing and the 10.0 mg/kg treatment group at 1.0 hour after dosing. The control activity level for the 0.5 hour time point was apparently excessively high and affected the determination of inhibition levels. The rbc ChE activity level was reduced only for the 10 mg/kg treatment group with activities 74.1 and 65.0% of control activity at 0.5 and 1.0 hours after dosing. These study results provide a range of times and concentration levels at which cholinesterase activities in the blood are affected via oral uptake of the test material and are useful in monitoring exposure scenarios. Study **supplemental**. (Moore, 11/5/99)

52610-070; 170859; "Cholinesterase Inhibition after Dermal Administration to Rats"; (R.D. Morrison et. al.; Rohm and Haas Company, Toxicology Department, Spring House, PA; Report No. 88R-184; 5/30/89); In Phase 1, 10 and 45 female rats were dermally treated with 0 or 50.6 mg a.i./kg b.w. of RH-57,988 4EC (lot no. LC-02-31, a.i.: 46%), respectively. Five treated rats/time point were euthanized at 1, 2, 4, 6, 8, 10, 12, 24, and 48 hours after dosing and plasma and red blood cell cholinesterase (ChE) activities were assayed. Maximal inhibition of plasma and rbc ChE was noted at 4 (52.8% of control) and 2 (78.7% of control) hours after dosing, respectively. In Phases 2 and 3, 5 females or 5 males were treated dermally with 0, 1.0, 5.0, 20.0, 50.0 or 100.0 (F only) mg a.i./kg b.w. of RH-57,988 4EC and euthanized 4 hours after dosing. In Phase 2, significant plasma ChE inhibition was noted at 50 and 100 mg a.i./kg (61.7 and 50.3% of control, respectively). Maximal inhibition of rbc ChE was noted at 20 mg a.i./kg (66.0% of control). Treatment at the two higher dose levels resulted in less inhibition (50: 84.4% of control and 100: 76.0% of control) which indicated a questionable dose response. In Phase 3, maximal plasma ChE inhibition was noted for the 50 mg a.i./kg group of 81.0% of control. For rbc ChE, the activity among the treated groups ranged from 86.3 (20 mg a.i./kg) to 91.6% (1 mg a.i./kg) of control. Overall, the results are indicative of dermal uptake of the test material at the higher doses tested, based upon plasma ChE activity levels. Study **supplemental**. (Moore, 11/4/99)

52610-069; 170858; "The Effects of Atropine on the Oral Toxicity of RH-7988 in Male Mice"; (K.M. Krzywicki and G.A. Hazleton; Rohm and Haas Company, Research Laboratories, Spring House, PA; Report No. 86R-158; 8/29/86, revised, 9/11/89); Ten male mice/group were dosed orally by gavage with 0 (corn oil), 25, 50 and 75 mg/kg of RH-7988 (purity: 97.0%) with or without atropine treatment. Atropine, 100 mg/kg, was administered by ip injection at 0, 2, 4, and 8 hours and orally by gavage 10 hours after dosing with the test material. In the groups not treated with atropine, mortality was 0: 0/10, 25: 0/10, 50: 2/10 and 75: 8/10. For the atropine-treated animals, one animal died in the 75 mg/kg group. Clinical signs for the animals not receiving the antidote included yellow stained urogenital area/diarrhea, fasciculations, salivation and ataxia. Except for fasciculations, these signs were largely

absent in the animals treated with atropine. However, these animals demonstrated atropine-related signs of dilated pupils, sluggishness and deep abdominal breathing. Heroic treatment with atropine resulted in enhanced survival of the study animals with less manifestation of treatment-related signs. Study **supplemental**. (Moore, 11/19/99)