CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
N-(((3,5-dichloro-2-fluoro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)amino)carbonyl)-2,6-diflurobenzamide

Chemical Code # 005816, DPN # 52905
SB 950 # NA
September 26, 2002
Revised: 7/29/03, 7/21/04, 8/22/05

I. DATA GAP STATUS

Combined (Chronic/onco), rat:	No data gap, acceptable, possible adverse effect
Chronic toxicity, dog:	No data gap, no adverse effects.
Oncogenicity, mouse:	No data gap, possible adverse effects
Reproduction, rat:	No data gap, possible adverse effects
Teratology, rat:	No data gap, no adverse effects
Teratology, rabbit:	No data gap, no adverse effects
Gene mutation:	No data gap, no adverse effects
Chromosome effects:	No data gap, no adverse effects
DNA damage:	No data gap, no adverse effects
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 218001 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T050822
P. Leung (10/31/02); T. Moore (7/29/03); M. Silva (7/21/04, 8/22/05)

NOTE: XDE-007 is also called XR-007 and X55007

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

**** 52905 - 0059 (6 volumes) 218084** "XDE-007: Two-year Dietary Chronic Toxicity/Oncogenicity and Chronic Neurotoxicity Study in Fischer 344 Rats," (Yano, B.L., Dryzga, M.D., Thomas, J.; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Laboratory Project Study ID: 011168; 4.22.05). Noviflumuron ((N-[3,5-dichloro-2-fluoro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-N'-(2,6-difluorobenzoyl)urea; XDE-007, 97.9% pure) was fed in diet to Fischer 344 rats (75/sex/dose) for 90 days, 1 or 2 years at 0, 0.1, 1.0, 75 or 300 mg/kg/day. Subchronic toxicity was assessed after 90 days of treatment on 10/sex/dose at 0 and 1.0 mg/kg/day doses only. At 1 year,10/sex/dose (chronic toxicity group), and 5/sex/dose (chronic neurotoxicity group) were necropsied. The remaining 50/sex/dose were necropsied after 2 years (oncogenicity group). Chronic NOEL = 1.0 mg/kg/day (At $\geq$ 75 mg/kg/day there were decreased body weights and body weight gains along with increases or decreases in absolute and/or relative organ weights (liver, kidney, brain, heart, adrenal, testes, spleen, epididymides were affected) at 12 and/or 24 months. Skin/tail papules and pustules were observed in males (300 mg/kg/day) and females ($\geq$ 75 mg/kg/day) in the second year. Females showed an increase in phthisis bulbi at 300 mg/kg/day. Prothrombin time in males and cholesterol levels in females were increased at $\geq$ 75 mg/kg/day at 24 months. ALP activities were increased in both sexes at $\geq$ 75 mg/kg/day at 24 months. Urine specific gravity was decreased (both sexes at $\geq$ 75 mg/kg/day) and urine volume was increased (M 300 mg/kg/day and F $\geq$ 75 mg/kg/day) at 24 months. Lung inflammation, hepatocytic hypertrophy (both sexes $\geq$ 75 mg/kg/day), mineralization of renal pelvic epithelium (M $\geq$ 75 mg/kg/day), epididymal aspermia (M $\geq$ 75 mg/kg/day), atrophy of seminiferous tubules (300 mg/kg/day), tail hyperkeratosis +/- inflammation (both sexes 300 mg/kg/day), tail tip necrosis (M 300 mg/kg/day) and hyperplasia of renal pelvic epithelium (M 300 mg/kg/day) were observed at 24 months. Leukemia was decreased in both sexes at $\geq$ 75 mg/kg/day at 24 months.) **Possible adverse effect:** Hepatocellular adenomas (benign, M 300 mg/kg/day), uterine stromal polyps (F $\geq$ 75 mg/kg/day), and liver foci (M $\geq$ 75 mg/kg/day) were increased at 24 months. Acceptable. Silva, 8/19/05

CHRONIC TOXICITY, RAT

Subchronic Study:

008; 186500; "XR-007: 4-Week Dietary Toxicity Study in Fischer Rats" (Lick, S.J. et al., Health & Environmental Research Laboratories, The Dow Chemical Company, Midland, MI, Laboratory Project Study ID 971106, 10/9/97). XR-007 (Lot # DECO-615-112, purity = 99.6%) was admixed to the feed and fed to 5 Fischer 344 rats per sex per dose at dose levels of 0, 1, 10, 100, 500, or 1000 mg/kg/day (0, 1.0, 10.4, 101.4, 512.6, 1029.1 mg/kg/day, respectively for males and 0, 1.1, 10.9, 105.1, 520.6, 1055.6 mg/kg/day, respectively for females) for 4 weeks. No mortalities occurred. No treatment-related clinical signs were observed. A treatment-related increase in mean relative liver weight was observed in both sexes at 500 and 1000 mg/kg/day. Microscopic examination revealed treatment-related hepatocellular hypertrophy (centrilobular) in males at 1000 mg/kg/day and in females at 500 and 1000 mg/kg/day. **No adverse effects.** NOEL (M) = 101.4 mg/kg/day and NOEL (F) = 105.1 mg/kg/day (based on an increase in mean relative liver weight and hepatocellular hypertrophy). **Supplemental** (because only 5 animals per sex per dose were used, the animals were treated for only 4 weeks, no ophthalmological examinations were conducted, and no analysis of the dosing material was conducted). (Corlett, 10/3/02)

CHRONIC TOXICITY, DOG

Subchronic Study:

** 52905 - 0054 204646 "XDE-007: 90-Day Dietary Toxicity Study with 28-Day Recovery in Beagle Dogs," (Stebbins, K.E., Thomas, J., Baker, P.C.; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Laboratory Project Study ID #: 011194; 5/9/03). XDE-007 (98.4% pure) was fed in diet to Beagle dogs (4/sex/dose) for 90 days at 0, 0.003, 0.3 or 3.0% (equivalent to 0, 0.913, 115 and 1040 mg/kg/day - Males and 0, 1.06, 113 and 1150 mg/kg/day - females). Recovery groups (4/sex/dose) were fed XDE-007 in diet at 0 or 3% for 90 days, then were maintained on control diet for 28 days. All animals survived the study. There were no treatment-related effects in body weight and food consumption. NOEL = 0.003%; equivalent to 0.913 mg/kg/day - Males; 1.06 mg/kg/day - Females (There was a statistically significant decrease in RBC (males) and increase in platelets at 6 and 13 weeks in both sexes of dog at $\geq$ 0.3% XDE-007 (RBC reversed after recovery). Mean HGB and HCT were decreased in males at 6 and 13 weeks at $\geq$ 0.3% XDE-007 (not statistically significant) and was reversed after recovery. Mean MCV was decreased in both sexes at 6 and 13 weeks at $\geq$ 0.3% XDE-007 (not statistically significant) and was reversed in males but not females after recovery. Neutrophils were increased and lymphocytes were decreased (not statistically significant) in males at 6 and 13 weeks at 3.0% XDE-007 (reversed at recovery). Relative liver in both sexes and relative kidney weights in males were increased at 3.0 mg/kg in the main dosing groups (reversed at recovery). There was an increased incidence in bone marrow hyperplasia (erythroid cell) at $\geq$ 0.3% XDE-007 in the main dose group that persisted after recovery.) No adverse effect. Acceptable. (M. Silva, 6/21/04)

Chronic Study:

** 52905 - 0055 210330 "XDE-007: One-Year Dietary Toxicity Study in Beagle Dogs," (Stebbins, K.E., Day, S.J., Thomas, J.; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Laboratory Project Study ID #: 142640; 3/5/04). XDE-007 (97.9% pure) was fed in diet to Beagle dogs (4/sex/dose) for 1 year at 0, 0.003, 0.03 or 0.225% (equivalent to 0, 0.74, 9.3 and 69 mg/kg/day - Males and 0, 0.94, 8.7 and 70 mg/kg/day - females). NOEL = 0.003% (0.74 mg/kg/day - Males; 0.94 mg/kg/day - Females) (Mean corpuscular volume in males (compared with controls at each time interval) and reticulocytes in both sexes were increased at 0.225% throughout the study. Both sexes at $\geq$ 0.225% had statistically significantly increased mean platelet count. There was a significant increase in ALP at 0.225% in females at 3 and 12 months. There was a statistically significant increase in absolute adrenal weights (analyzed across both sexes) at 0.225% without histological findings. There was an increased incidence in bone marrow erythroid hyperplasia (erythroid cell) at $\geq$ 0.3% XDE-007 (males, 0, 0, 4, 4 --each dose level) and at 0.225% (females: 0, 0, 0, 1 --each dose level). There was a dose-related increase in severity of stomach mucosal lymphoid hyperplasia in the fundus and pylorus in both sexes at $\geq$ 0.3%.) No adverse effect. Acceptable. (M. Silva, 6/21/04).

ONCOGENICITY, MOUSE

Subchronic Study:

007; 186499; "XDE-007: 28-Day Dietary Toxicity Study in CD-1 Mice" (Yano, B.L. and Day, S.J., Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI, Laboratory Project Study ID 001248, 6/12/01). XDE-007 (Lot, Reference No. F0031-148, TSN102332, purity = 98.4%) was admixed to the feed and fed to 5 CD-1 mice per sex per dose at dose levels of 0, 10, 100, 500, or 1000 mg/kg/day (0, 10.8, 110, 538, 1060 mg/kg/day, respectively for males and 0, 11.2, 113, 504, 1140 mg/kg/day, respectively for females) for 28 days. No mortalities occurred. No treatment-related clinical signs were observed. Treatment-related increases in mean platelet level and mean cholesterol level were observed in both sexes at 100, 500, and 1000 mg/kg/day. A treatment-related increase in mean relative liver weight was observed in males at 100, 500, and 1000 mg/kg/day and in females at 500 and 1000 mg/kg/day. Microscopic examination revealed treatment-related hepatocellular hypertrophy with altered tinctorial properties

(centrilobular/midzonal to panlobular) in males at 500 and 1000 mg/kg/day and very slight vacuolation (consistent with fatty change) of the periportal hepatocytes in males at 500 and 1000 mg/kg/day and in females at 1000 mg/kg/day. **No adverse effects.** NOEL (M) = 10.8 mg/kg/day and NOEL (F) = 11.2 mg/kg/day (based on increases in mean platelet and mean cholesterol levels). **Supplemental** (because only 5 animals per sex per dose were used and because the animals were treated for only 28 days). (Corlett, 9/30/02)

Definitive Study:

**** 52905 - 0058 (3 volumes) 218001** "XDE-007: 18-Month Oncogenicity Study in CD-1 Mice," (Johnson, K.A.; Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; 4/25/05). XDE-007 technical (N-[3,5-dichloro-2-fluoro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-N'-(2,6-difluorobenzoyl)urea), 97.9% pure) was fed in diet to CD-1 mice (50/sex/dose) at 0, 0.5, 3 (males only), 30 and 100 (females only) mg/kg/day for up to 18 months. Systemic NOEL = 3 mg/kg/day (males) and 30 mg/kg/day (females) There was an increase in mortality for females at 100 mg/kg/day (M: 56% vs 32%). There was an increased incidence in tonoclonic convulsions (primarily in females) at the high dose. There was an increase in relative and absolute liver weights in females at $\geq$ 30 mg/kg/day. Males had an absolute liver weight increase at 30 mg/kg/day and a relative increase at $\geq$ 3 mg/kg/day. Both sexes showed an increase in all severities of liver hypertrophy (hepatocytic, centrilobular/midzonal, diffuse) at 30 mg/kg/day (male) and at 100 mg/kg/day (female). There was an increased incidence in evidence of inflammation in lung in both sexes at the high dose. **Possible adverse effect indicated:** There was an increased incidence in hepatocytic adenomas and in hepatocytic adenomas plus carcinomas (slight in males, statistically significant in females) at 30 mg/kg/day (5, 7, 4, 12 of 50) and 100 mg/kg/day (F: 0, 1, 0, 4** of 50 by Peto's mortality adjusted statistics). Females showed an increased incidence in lung carcinomas (non-metastatic) at 100 mg/kg/day (0, 0, 1, 2** by Peto's statistics). Acceptable. M. Silva, 8/2/05

REPRODUCTION, RAT

52905 - 0057 210840 "XDE-007: One-Generation Dietary Reproduction Toxicity Study With Cross-Fostering in CD Rats," (Marty, M.S., Zablotny, C.L., Liberacki, A.B., Yano, B.L.;Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI; Laboratory Project Study ID: 011221; 3/23/04). XDE -007 (97.9% pure) was fed in diet to Crl:CD (SD) IGS BR rats (30/sex/dose) at 0, 0.5, 5 and 100 mg/kg/day continuously from pre-mating (10 weeks) of parental generation, through breeding (2 weeks), gestation (3 weeks) and lactation through weaning of F1 offspring. Although designed as a 2 generation reproduction study, it was terminated after 1 generation due to excessive F1 pup mortality at 100 mg/kg/day. Subsequently a Cross-fostering Study was performed to determine whether the decreased survival in pups from XDE-007 treated P1 rats resulted from *in utero* or lactational exposure. Parental Systemic NOEL = 0.5 mg/kg/day (Tonoclonic convulsions were observed in 2/30 males and 1/24 females at 100 mg/kg/day. Males had statistically significantly decreased food consumption from day 8 until termination at 100 mg/kg. Females had statistically significantly decreased food consumption during lactation. Male P1 premating body weights were statistically significantly decreased at 100 mg/kg/day. Mean gestational body weight gain in females was statistically significantly decreased GD 0 - 7 (but not GD 0 - 21) and lactation days 7 - 13 at 100 mg/kg/day.) Reproduction and Fertility NOEL = 0.5 mg/kg/day (P1 female gestation survival was decreased and gestation length was increased at 100 mg/kg. Pup NOEL = 0.5 mg/kg (F1 survival was drastically decreased throughout lactation at 100 mg/kg. Clinical effects were increased in pups at 100 mg/kg, primarily tonoclonic convulsions, no milk in stomach, entire litter loss and death. Pup weights were statistically significantly decreased at 100 mg/kg. Cross-fostering data indicate the proximate toxicant (either XDE-007 and/or its metabolites) led to decreased pup survival through the maternal milk and not through exposure *in utero*. Possible adverse effect indicated: excessive neonatal/pup mortality and increased tonoclonic convulsions in pups (17/24 litters) at 100 mg/kg. These data are supplemental. M. Silva, 7/20/04

**** 52905 - 0056 210839** "XDE-007: Two-Generation Dietary Reproduction Toxicity Study in CD Rats," (Carney, E.W., Zablotny, C.L., Liberacki, A.B., Yano, B.L.;Toxicology & Environmental

Research and Consulting, The Dow Chemical Company, Midland, MI; 3/22/04). XDE -007 (97.9% pure) was fed in diet to Crl:CD (SD) IGS BR rats (30/sex/dose/generation) at 0, 0.5, 5 or 25 mg/kg/day continuously from pre-mating of parental generation 1 (P1) through weaning of offspring through 2 generations (2 matings for P2 generation) to F2b weaning. Parental Systemic NOEL = 5.0 mg/kg/day (There were increased absolute and relative liver weights in both sexes of P1 and P2 adults at 25 mg/kg and in P1 female liver weights at 5.0 mg/kg. P1 male relative kidney weights, P2 female absolute kidney weights, P2 male relative spleen and thyroid weights and P2 female absolute and relative adrenal weights were increased at 25 mg/kg.) Reproduction and Fertility NOEL = 5.0 mg/kg/day (There was an increased proportion of abnormal P2 sperm at 25 mg/kg. Although not statistically significant, there were decreased mating and fertility indices in both sexes of P2a and P2b adults at $\geq$ 5.0 mg/kg.) Pup NOEL = 5.0 mg/kg (There was a decrease in F1 survival (not statistically significant) on lactation days 14 and 21 at 25 mg/kg. F2a pups had statistically significantly decreased survival of lactation days 7, 14 and 21at 25 mg/kg. Mean F2a litter size was statistically significantly decreased on lactation days 14 and 21 at 25 mg/kg. Mean F1 pup weights were statistically significantly decreased at 25 mg/kg on lactation days 1, 14 and 21. Mean F2a male pup weights were statistically significantly decreased on lactation days 1 and 21 and F2a female pup weights were decreased on lactation day 21 at 25 mg/kg. F2a male weanlings had statistically significantly decreased body weights, relative brain and absolute spleen weights at 25 mg/kg. Neonates at 25 mg/kg in both F1 and F2 generations showed tonoclonic convulsions, as well as in 1 litter of F2a at 5.0 mg/kg. P2 females (1, 1, 5 and 7 at 0, 0.5, 5.0 and 25 mg/kg, respectively) failed to litter, so the female is considered to be an affected sex.) Possible adverse effects on reproduction, fertility and pup survival, along with numerous other toxicologically relevant effects. M. Silva, 6/18/04

TERATOLOGY, RAT

52905-011 186503, “XDE-007: Oral Gavage Developmental Toxicity Study in CD Rats”, (M.S. Marty and C.L. Zablotny, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI., Report # 991040, 14 September 2000). 25 time-mated female CD rats received XDE-007 technical (98.6% purity) by gavage at 0 (0.5% methylcellulose), 250, 500, and 1000 mg/kg/day on gestation days 6 through 20. Implantation loss at the high dose was slightly increased relative to study controls but well within the range of historical control values. There were no affects on clinical signs, bodyweight, food consumption or organ weights (liver, kidney, uterus). **No developmental toxicity, no adverse effects. Maternal and Developmental NOEL = 1000 mg/kg/day. **Acceptable**. (Green and Gee, 10/4/02).

52905-009 186501, “XR-007: Whole Embryo Culture Teratogenicity Screen”, (E. W. Carney, Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, MI, Report # 971083, 31 July 1997). Seven non-pregnant female rats (serum donors) received 1000 mg/kg/day of the test article (XR-007, 98%) in 0.5% Methocel by gavage for 3 consecutive days. Four hours after the last dose, rats were exsanguinated and their blood centrifuged to obtain serum. Six control rats were similarly treated with vehicle and bled. Sera were heat inactivated (30 min. at 56°C), sterile filtered, and stored (-80°C) for 3 days. Rat conceptuses with intact amnion and visceral yolk sac, but with Reichert’s membrane removed, were explanted from a separate group of untreated, timed-mated rats on the afternoon of gestation day 9. Embryos were cultured in 100% sera from the control or treated donors, and, after 48 hours, evaluated for viability, growth, and morphology. All serum donors appeared normal throughout the test period. Bodyweight gains were similar in control and treated animals. 12/12 treated and 11/12 control embryos had a beating heart and visible yolk sac circulation. Statistically significant increases in crown-rump length and somite number for treated embryos were reportedly due to a lower than usual growth rate in control embryos. Morphological abnormality was limited to an abnormal curvature of the anterior neural tube which distorted the head in one treated embryo (8.3%). No historical control data. This is **supplemental** information. (Green and Gee, 10/3/02).

TERATOLOGY, RABBIT

52905-010 186502, “XDE-007: Oral Gavage Developmental Toxicity Study in New Zealand White Rabbits”, (E.W. Carney, *et al*., Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI., Report # 991084, 15 March 2000). Twenty-five time-mated New Zealand White female rabbits received XDE-007 (98.6% purity) by gavage at 0 (0.5% methylcellulose), 250, 500, and 1000 mg/kg/day on gestation days 7 through 27. Absolute and relative maternal liver weights were slightly increased in treated groups relative to controls (not statistically significant). Implantation loss in treated groups was slightly higher than study controls but not statistically significant or dose-related. Fetal weight, live litter size, and incidence of malformations/variations were not affected. Maternal bodyweights, food consumption, and other parameters were comparable. **No adverse effects. Maternal and Developmental NOEL = 1000 mg/kg/day. **Acceptable**. (Green and Gee, 10/4/02).

GENE MUTATION

52905-013 186505, “*Salmonella - Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with XDE-007". (Michael S. Mecchi, Covance Laboratories Inc., Vienna, VA., Covance Study # 22129-0-422OECD, Dow Study ID. 011001,** 20 June, amended 26 July and 14 August 2001). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed to XDE-007 technical (98.4% purity), in the presence and absence of S9, at 0 (DMSO), 33.3, 100, 333, 1000, 3330, and 5000 μg/plate using the preincubation method. Cultures were treated, and preincubated for 20 ± 2 minutes at 37°C. Molten agar was then added, tubes were revortexed, and contents plated (petri dishes) and incubated for 52 ± 4 hours. Background lawn status and precipitation were evaluated. Positive controls were functional. No increase in the reversion frequency. **Acceptable.** (Green and Gee, 10/3/02).

52905-015 186507, “Evaluation of XDE-007 in the Chinese Hamster Ovary Cell/Hypoxanthine-Guanine-Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay”, (V.A. Linscombe and D.J. Beuthin, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI., Report # 011076, 26 July 2001). Chinese hamster ovary cells (CHO-K_1-BH_4) were exposed (4 hours) in duplicate cultures (1 x 10^6 cells/dish), in the presence and absence of S9, to XDE-007 technical (98.4% purity) at 0 (DMSO), 12.5, 25, 50, 100, and 200 μg/ml in the main assay and at 0, 6.66, 20, 66.6, and 200 μg/ml in the confirmatory assay. Cultures were trypsinized at the end of treatment and replated in duplicate at a density of 1 x 10^6 cells/100 mm dish for phenotypic expression (7 to 9 days). In addition, 200 cells/60 mm dish (3 dishes/replicate) were also plated and incubated 6-8 days to allow colony formation to determine toxicity. At the end of expression, cells were plated in 10 replicate dishes per initial concentration with 6-thioguanine for selection. Cloning efficiency was also determined. No increase in forward gene mutation at the hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) locus. Positive controls were functional. **Acceptable. (Green and Gee, 10/3/02).

CHROMOSOME EFFECTS

52905-009 186504, “Screening of XR-007 in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes”, (V. Ann Linscombe, Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, MI., Report # 971069, 16 June 1997). Lymphocytes from male Sprague-Dawley rats (100 µl of whole blood/ml of complete culture medium) were exposed in duplicate cultures to XR-007 at 0 (1% dimethylsulfoxide), 0.5, 1.67, 5.0, 16.7, 50, 167, and 500 µg/ml for 4 (+20 hours) and 24 hours in the presence and absence of S9 respectively. Treatment began approximately 48 hours after initiation of the cell cultures. Aberration results were reported for lymphocytes treated at 50, 167, and 500 µg/ml. These levels were chosen based on mitotic indices. An increase in clastogenic activity was not indicated. **Unacceptable**, upgradeable (description, justification, and discussion of assay performance and results; GLP). (Green and Gee, 10/3/02).

52905-016 186508, "Evaluation of XDE-007 in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes", (V.A. Linscombe, *et al.,* Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI., Report # 011071, 23 July 2001). Lymphocytes from male Sprague-Dawley rats (outbred Crl: CD BR) (stimulated for 48 hours with PHA) were exposed to XDE-007 (98.4% purity) in duplicate cultures for 4 hours, in the presence and absence of S9, at 0 (1% DMSO), 3.13, 6.25, 12.5, 25, 50, 100, and 200 μg/ml in the initial assay with harvest 20 hours later. In the confirmatory assay, continuous treatment for 24 hours at the same concentration levels (as the initial assay) was used in the absence of S9. Cultures were exposed 4 hours at 0, 12.5, 25, 100, and 200 μg/ml in the presence of S9. Cultures in both assays were harvested 24 hours after treatment initiation. Based on the mitotic indices, treatment levels 25, 100, and 200 μg/ml were chosen for evaluation (100 cells per replicate) of chromosomal damage. Mitotic indices indicated moderate toxicity at higher concentrations. Positive controls were functional. No increase in chromosomal aberrations. **Acceptable. (Green and Gee, 10/2//02).

DNA DAMAGE

52905-014 186506, ""Evaluation of XDE-007 in the *In Vivo* Mouse Micronucleus Assay", (Gregory L. Erexson, Covance Laboratories, Inc., Vienna, VA., Covance Study # 22129-0-455OECD and Dow Study # 011077, 9 August 2001). 7 male Crl:CD-1®(ICR)BR mice per group received XDE-007 technical (98.4%) once daily on 2 consecutive days at 0 (0.5% methylcellulose), 500, 1000, and 2000 mg/kg/day. Use of a single sex (male) was based on a preliminary test at these same doses in 4/sex/dose. There were no mortalities due to XDE-007 in either trial. Bone marrow from 6 per group was sampled 24 hours after the last dose. For micronuclei, 2000 PCEs per animal were examined. The PCE/NCE also was determined. There was no significant decrease in PCE/NCE. No increase in micronuclei. **Acceptable. (Green and Gee, 10/3/02).

NEUROTOXICITY

Not required at this time.

SUPPLEMENTAL, INSECT METABOLISM

52905-025 186517, "Kinetics of Uptake, Clearance, Transfer, and Metabolism of Hexaflumuron and XR-007 in Termites (*Reticulitermes flavipes)*", (J.J. Sheets, *et al.*, Dow AgroSciences LLC, Indianapolis, IN., Report # DAI0149, 2 June 1999). This assay compared the uptake, clearance, transfer, and metabolism of the radiolabelled chitin synthesis inhibitors hexaflumuron and XR-007 in termites (*Reticulitermes flavipes*).

The termite holding apparatus for the uptake kinetics phase consisted of a cylindrical plexiglass chamber (5 cm wide x 4 cm high) containing a 2:1 mixture of sand and vermiculite. Coupled to the chamber through a piece of Tygon tubing (2 mm x 10 cm) was a single feed chamber of identical dimensions containing the cellulose diet (filter paper). Cellulose was treated with [^{14}C] labeled hexaflumuron or XR-007 at 0.1% and 0.5%. Hexaflumuron and XR-007 were labeled in different rings and mixed in equal proportions (pg. 6). Radio chemical purity of all 4 samples was > 95% (pg. 7). Each apparatus contained 100 termites. The amount of radioactivity contained in each live termite was measured by scintillation counting at 2, 6, 10, 15, 20, 30, and 40 days after treatment began. At each time point, 5 groups of 5 termites each were sampled and digested with 200 μl of Protosol™. Only live termites were measured.

For clearance rate measurements, 1000 termites were placed in a petri dish and force fed cellulose disk diets containing either 0.1% or 0.5% [^{14}C] XR-007 or [^{14}C] hexaflumuron. Termites were then transferred in groups of 100 into an apparatus consisting of a single feeding chamber with untreated paper disks connected with a plastic tube to a housing chamber. 5 groups of 5 termites each were sampled at 0, 16, 30, 48, 72, 144, and 240 hours after removal from the treatment diet. Termites

were placed into 4 ml plastic scintillation vials and digested in 200 μl Protosol™. Radioactivity content of the termites was determined by scintillation spectrophotometry.

For trophallaxis transfer evaluation, approximately 1000 termites were placed in each of four petri dishes (18 cm diameter) containing paper diet treated with a 0.1% acetone solution of Nile Blue, 2.6 μl/mg/dry paper and with either 0.1% or 0.5% w/w of [^{14}C] XR-007 or [^{14}C] hexaflumuron. Termites were allowed to feed on the treated paper for 7 days. The blue dyed termites were then mixed with untreated (white) termites in an apparatus consisting of a housing and feeding chamber. The feeding chamber contained untreated filter paper. Treated/untreated mixing ratios were 1:5, 1:10, and 1:20, with total number of termites per apparatus of 210. White termites (untreated) were measured (scintillation counting after Protosol™ digestion) for the amount of radioactivity in their bodies at 8 hours, and at 1, 2, 4, 7, and 10 days after mixing of the 2 termite populations.

For internal dose response, toxicity was measured by placing groups of 100 termites (*R. flavipies*) into holding apparatuses having a single cellulose feeding container. Groups consisted of 4 radiolabelled concentrations of [^{14}C] XR-007 or [^{14}C] hexaflumuron, 0.00195%, 0.0065%, 0.0195%, and 0.065%, one unlabelled dose, 0.00065%, and 10 control (untreated) groups. Five replicates were done for each concentration. Mortality was recorded 15, 20, 25, 30, 45, and 60 days after initial exposure to the treated paper. At 20, 30, 40, 50, and 60 days after exposure initiation, two live termites for each apparatus were sampled for radioactivity. The grading of the observations consisted of 3 descriptions: **OK** = normal, **Affected** = moving slowly, **Collapsed** = no foraging and > 80% morality, representing 0%, 50%, and 100% mortality respectively. When the population in an apparatus was judged to have collapsed, any live termites were collected and sampled for radioactivity. The amount of XR-007 and hexaflumuron in the non-labeled group, 0.00065%, was estimated by linear regression from the labeled group data.

To evaluate termite metabolism of the test compounds, the sand/vermiculite mixtures within the housing chamber of termites fed 0.1% and 0.5% [^{14}C] XR-007 or [^{14}C] hexaflumuron for 40 days were extracted 3 times each with acetone, and filtered through a 0.45 μm membrane. The solvent was evaporated and the residue suspended in a small volume of acetone and spotted onto thin-layer chromatography (TLC) plates for development.

Results

XR-007 was more toxic and faster acting than hexaflumuron in all the experiments performed in this study. Internal uptake of XR-007 was generally less than hexaflumuron, especially at higher concentrations, yet the time to kill and the minimum toxic concentration were lower. Hexaflumuron was cleared from termites in a first order process with a half life of 8-9 days. The half life of XR-007 was 29 days for 0.5% and 191 days for 0.1%. Neither compound was metabolized by the termites. Both XR-007 and hexaflumuron were transferred horizontally to other termites by trophallaxis in a highly efficient manner (near 100% efficiency of transfer) during the first 8 hours of association. With increasing time, the amount of XR-007 inside of termites dropped to a steady state by about day 4 while hexaflumuron bodily concentrations continued to fall, eventually reaching zero.

Supplemental information. (Green and Gee, 10/02/02).

52905-026 186518, "Uptake Rate of [^{14}C] Hexaflumuron Force-Fed to Groups of *Reticulitermes flavipes*", (J.J. Sheets, *et al.*, Dow AgroSciences LLC, Indianapolis, IN., Report # DEI0376, 2 May 1997). Evaluation of the uptake of the [^{14}C] labeled chitin synthesis inhibitor hexaflumuron in termites (*Reticulitermes flavipes*) was performed. Results were compared with limited previous data obtained with *R. santonensis*.

The termite cage apparatus consisted of 3 cylindrical plexiglass chambers 5.0 cm in diameter, and 4.0 cm high. The center housing chamber contained a half volume mixture of sand and vermiculite (2:1 v/v), and was connected with small Tygon tubing (2 mm in diameter, 10 cm long) to 2 additional feeding chambers which contained hexaflumuron treated paper. 200 termites per apparatus were introduced into the housing chamber and allowed to forage and make contact with the connected feeding chambers. After 3-4 days of acclimation, paper disks treated with either 0.1% or 0.5% w/w

[^{14}C] hexaflumuron (Lot # F0032-149, sp. act. of 30.6 mCi/mmole, > 99% purity) were placed in the feeding chambers. 10 termites from each apparatus (conducted in duplicate) were collected from the feeding chambers daily after exposure to treated paper disks and placed in scintillation vials and treated with Protosol. The amount of radioactivity contained in the termites was determined (5 minutes) by scintillation spectrophotometry. The amount of paper consumed at each time point was estimated by visual inspection. The amount of hexaflumuron and its metabolites contained in each termite was calculated based on the known specific activity of the radiolabeled material diluted with non-labeled hexaflumuron.

At both treatment levels, the amount of uptake of radioactivity initially increased rapidly with time and then leveled off at a maximum steady state concentration. The initial rate of hexaflumuron uptake (calculated for the first 7 days of the experiment) at 0.1% was 12.7 ng/termite/day with a steady state level of 110-130 ng/termite achieved after 14-18 days of exposure. Termites force-fed the diet of 0.5% hexaflumuron had an initial uptake rate of 55 ng/termite/day with a steady state concentration of 320 ng/termite after 9-12 days.

A direct comparison of the rate of uptake of [^{14}C] hexaflumuron for the two termite species could not be made due to the difference in techniques used to measure the radioactivity. In the *R. flavipes* assay, the termites were solubilized with Protosol to eliminate any quenching of the radioactivity by the insect proteins during scintillation counting, *R. santonensis* were not. Time to maximum steady state concentration was compared and observed to be 2 times faster for *R. flavipes* versus *R. santonensis* (14-18 days versus 32 days).

No attempt was made to measure the location of hexaflumuron in or on the body of termites, rate of elimination or characterization of the radioactivity.

Supplemental information. (Green and Gee, 10/02/02).