SUMMARY OF TOXICOLOGY DATA

Mesotrione

Chemical Code # 6069, Tolerance # 53157
SB 950 # NA

Original: March 6, 2012
Revised: July 6, 2012

I. DATA GAP STATUS

<table>
<thead>
<tr>
<th>Category</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic toxicity, rat</td>
<td>No data gap, possible adverse effect</td>
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<tr>
<td>Chronic toxicity, dog</td>
<td>No data gap, no adverse effect</td>
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<tr>
<td>Oncogenicity, rat</td>
<td>No data gap, no adverse effect</td>
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<tr>
<td>Oncogenicity, mouse</td>
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<tr>
<td>Reproduction, rat</td>
<td>No data gap, possible adverse effect</td>
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<tr>
<td>Teratology, rat</td>
<td>No data gap, no adverse effect</td>
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<tr>
<td>Teratology, rabbit</td>
<td>No data gap, no adverse effect</td>
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<tr>
<td>Gene mutation</td>
<td>No data gap, no adverse effect</td>
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<tr>
<td>Chromosome effects</td>
<td>No data gap, possible adverse effect</td>
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<tr>
<td>DNA damage</td>
<td>No data gap, no adverse effect</td>
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<tr>
<td>Neurotoxicity</td>
<td>No data gap, no adverse effect</td>
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</table>

Toxicology one-liners are attached.

All record numbers through 265534 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
## indicates a study on file but not yet reviewed.
File name: T120706
Revised by T. Moore, 7/6/12
II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

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

COMBINED, RAT

53157-0037 257993, 831; “ZA1296: 2 year dietary toxicity and oncogenicity study in rats”, rat; Central Toxicology Laboratory, Cheshire, UK SK10 4TJ, 12/16/97; Brammer, A.; Laboratory Project ID: Report #: CTL/P/5481, Study #: PR1001; ZA1296 (technical material), (batch P17, a light beige powder, 96.8% w/w purity) 0, 7.5, 100 and 2500 ppm was administered to 64 Alpk:APfSD (Wistar-derived) rats/sex by dietary route for 1 year (12 rats/sex/group at interim kill) or 2 (52/sex/group) years. In addition, 20 male and 20 female rats were exposed to 1 or 2.5 ppm ZA 1296 by dietary route for 2 years to investigate ocular toxicity. Mean doses received for males were: 0.06, 0.16, 0.48, 6.48 and 159.89 mg ZA 1296/kg/day, and mean values for females were 0.08, 0.19, 0.57, 7.68 and 189.48 mg ZA1296/kg/day, for nominal dietary levels of 1, 2.5, 7.5, 100 and 2500ppm ZA1296, respectively. The male groups fed ZA 1296 were terminated when survival reached 25%. Groups 2 and 3, fed 1 and 2.5 ppm, were terminated in weeks 92 and 93 while the remaining male groups and control were terminated in weeks 97 or 98. The female groups survived to scheduled termination and there was no evidence of an effect on mortality. Significant reduction in bodyweight was reported in males fed 1 and 2.5 ppm during second year of study (10 and 11% respectively, compared to controls, week 91). The treatment related ophthalmoscopic findings were seen in >7.5 ppm ZA 1296 treated male rats and the high dose group female rats. Increased eye keratitis was observed in > 100 ppm ZA 1296 treated female rats in microscopic findings. Increased liver weight, liver weight to body weight ratio, increased incidence of pale liver and hepatocyte fat vacuolation were observed in > 7.5 ppm ZA 1296 treated male rats. NOEL for male rats: < 1 ppm (0.06 mg/kg/day) due to decreased body weights; NOEL for female rats: 2.5 ppm (0.19 mg/kg/day) due to increased clinical signs of cloudy eyes in 7.5 ppm and up ZA 1296 treated rats. No carcinogenic potential. Acceptable. (Pan and Leung, 10/20/11)

CHRONIC TOXICITY, RAT

See Combined, Rat above

CHRONIC TOXICITY, DOG

53157-0039 257997, 831; “ZA1296: One year oral toxicity study in dogs”, dog; Central Toxicology Laboratory, Cheshire, UK SK10 4TJ, 11/6/97; Brammer, A.; Laboratory Project ID: Report #: CTL/P/5511, Study #: PD1051; ZA1296 (technical material), (batch P22, a light beige solid, 97.6% w/w purity) Groups (4/sex) of beagle dogs were exposed to 0, 10, 100, or 600 mg ZA 1296/kg/day for periods of at least one year. One female dosed 600 mg/kg/day was humanely killed in week 47 due to sudden onset of adverse clinical signs, including convulsions, hypothermia, a slow weak pulse and rapid weight loss. Microscopic findings revealed general lymphocytolysis. NOEL: 100 mg/kg/day due to mortality in the 600 mg/kg/day treated group. Acceptable. (Pan and Leung, 10/25/11)

ONCOGENICITY, RAT

See Combined, Rat above.

ONCOGENICITY, MOUSE

53157-0040 257999, 832; “ZA1296: 80 week carcinogenicity study in mice”, mouse; Central Toxicology Laboratory, Cheshire, UK SK10 4TJ, 11/5/97; Rattray, N.; Laboratory Project ID: Report #: CTL/P/5281, Study #: PM0983; ZA1296 (technical material), (batch P17, a light beige solid, 96.8% w/w purity). Groups of 55 male and 55 female C57BL/10J:CD-1 Alpk mice were fed diets containing 0, 10, 350 or 7000ppm ZA 1296 [mean test substance uptake for males/females: 1.4/1.8, 49.7/63.5 and 897.7/1102.9 mg/kg/day for 10, 350 and 7000ppm respectively] for a period up to 80
weeks. The animals in 7000 ppm ZA 1296 treatment group received 3500 ppm ZA 1296 for 7 weeks in the beginning of the study followed by 7000 ppm ZA 1296 for the rest of the study period. Slight body weight reduction was noted in 350 and 7000 ppm treated male mice and in 7000 ppm treated female mice. There was no treatment related changes in survival, clinical signs, hematology and post mortem examinations. No Observed Effect Level (NOEL): 10 ppm (1.4 mg/kg/day) for male mice and 350 ppm (63.5 mg/kg/day) for female mice due to body weight changes. No carcinogenicity. Acceptable. (Pan and Leung, 10/27/11)

CHRONIC TOXICITY, MOUSE

53157-0038 257995, 831; “ZA1296: One year dietary toxicity study in mice”, mouse; Central Toxicology Laboratory, Cheshire, UK SK10 4TJ, 11/12/97; Pinto, P.; Laboratory Project ID: Report #: CTL/P/5682, Study #: PM1062; ZA1296 (technical material), (batch P17, a light beige solid, 96.8% w/w purity) Eighteen groups (20/sex/time points) of mice were exposed to 0, 0, 10, 50, 350 or 7000 ppm ZA 1296 (mean test substance intake for males were: 1.5, 7.8, 56.2 and 1114.0 mg/kg/day for the 10, 50, 350 and 7000 ppm dose groups respectively; mean values for females were: 2.1, 10.3, 72.4 and 1494.5 mg/kg/day for the 10, 50, 350 and 7000 ppm dose groups respectively) for periods up to 3 months, 6 months or one year Slightly decreased body weight and food utilization were observed in 7000 ppm ZA 1296 treated mice. NOEL: 350 ppm for male and female mice due to changes in body weights and food utilization.

Acceptable. (Pan and Leung, 10/21/11)

REPRODUCTION, RAT

53157-0025 257955, 834; “ZA1296: Multigeneration study in the rat”, reproductive toxicology, rat; Central Toxicology Laboratory, Cheshire, UK SK10 4TJ, 12/10/97; Milburn, G.; Laboratory Project ID: Report #: CTL/P/5147, Study #: RR0691; ZA1296 (technical material), (batch P17, a light beige powder, 96.8% w/w purity) 0, 2.5, 10, 100 and 2500 ppm was administered to 3 generations of Alpk:APfSD (Wistar-derived) rats by dietary route. For each dose level the dose received during the pre-mating periods was approximately 0.3, 1.2, 12 and 300 mg/kg/day in the 2.5, 10, 100 and 2500 ppm groups respectively. Twenty six males and 26 females were selected for each dosing group for each generation. Every dosing group of the third generation rats was divided into two subgroups at week 14 after selection: recovery (14M/14F) and continuous treatment (12M/12F), with recovery group rats fed control diets while the continuous treatment group rats were kept on the same dietary exposures to ZA1296. Clinical observations, bodyweights and ophthalmoscopy were investigated for all the rats. Food consumption for adults, survival and developmental landmarks for offspring were investigated. Post mortem investigations included organ weights, macroscopic and selective tissue examinations. Most of the bodyweight and food consumption changes were limited to the high dose group adults except for the food consumption during lactation period, food utilization during the pre-mating period when both the 100 and 2500 ppm groups showed significant reduction from the control groups. Increased incidence of cloudy eyes for adults and offspring (except for the F3A litter-recovery) were observed in 100 and 2500 ppm groups. Ophthalmoscopic findings in adults and offspring (except for the F3A litter-recovery) were mainly observed in the 100 and 2500 ppm groups. Parental NOEL (M) = 2.5 ppm due to ocular histopathological and renal hydronephrosis changes. Parental NOEL (F) = 10 ppm due to changes in ocular histopathology. Offspring NOEL (M/F) = 10 ppm due to eye opacity. Developmental NOEL for males < 2.5 ppm due to delayed preputial separation time, for females = 2500 ppm due to no effect at the highest dose. Reproductive NOEL = 2.5 ppm due to reduced litter size.

Acceptable. (Pan & Leung, 9/30/11)
ID: CTL Study # 1356, Syngenta # 857-97, Supplement to Rec# 257955. Groups of 20 time-mated Alpk:APfSD (wistar-derived) rats were fed diets containing 0, 0.5% tyrosine, 1% tyrosine, 2% tyrosine, 2500 ppm ZA1296, 2500 ppm ZA1296/0.5% tyrosine, 2500 ppm ZA1296/1% tyrosine, and 2500 ppm ZA1296/2% tyrosine from day 1 of gestation until day 29 post partum termination. Clinical observations, food consumption and bodyweights for the parent females, clinical observations, number and sex, and bodyweights for litters were monitored throughout the study. Plasma tyrosine levels on gestation day 3 for parents and at termination for both parents and litters were measured and kidneys of selected litters were examined for bilateral pelvic dilatation. Animals fed diets containing 2500 ppm ZA1296/2% tyrosine were terminated by day 11 of gestation for humane reasons due to severe eye lesions. Eye lesions were also noted in the maternal animals and offspring in the groups fed 2500 ppm ZA1296/tyrosine 0.5 and 1%. Eye opaqueness was seen in maternal animals and offspring fed with 2500 ppm ZA1296 diets (including 0, 0.5 and 1% tyrosine). Maternal bodyweight reduction was seen in the 2500 ppm ZA1296/tyrosine 0.5% and 1% groups during gestation starting day 4 and during lactation starting day 5. Decreased food consumption was seen in these two groups throughout the gestation and lactation period and in 2500 ppm ZA1296 group during week 1 of gestation and week 3 of lactation. In the 2500 ppm ZA1296/tyrosine 0.5% and 1% treated litters, the following observations were noted: increase incidence of whole litter loss, decreased proportion of pups born alive, decreased proportions of litters with all pups born live, decreased litter size, decreased proportion of pups surviving to day 22, and decreased proportions of litters with all pups surviving to day 22. There was a slight reduction of litter size and a significant reduction of proportions of pups surviving to day 22 in the litters fed diets containing ZA1296 alone. There was a slight reduction of proportions of pups surviving to day 22 in the litters fed tyrosine (0.5, 1 and 2%) alone. Plasma tyrosine concentrations increased markedly in the 2500 ppm ZA1296/tyrosine (0.5, 1 and 2%) treated maternal animals and pups over those fed with tyrosine alone (0.5, 1, and 2%). Incidence of kidney bilateral pelvic dilatation in the pups increased in all ZA1296 treated groups, with slight increase in the ZA1296 2500 ppm/tyrosine (0.5 and 1%) groups as compared to ZA1296 alone. Study Supplemental. (Pan and Leung, 7/25/11)
least 8 weeks of pre-mating period. Decreased food consumption during lactation period for both F0 and F1 adults were observed in the 7000 ppm groups. Body weight decrease was observed in 7000 ppm and 1500 ppm ZA1296 treated F0 adult during lactation period, in F1 male and female pups and adults during pre-mating period, gestation period and lactation period, and in F2 male and female pups. Decreased body weight was seen also in >10 ppm ZA1296 treated F1 male and female pups on days 22 and 29. Increased incidence of eye opaqueness was seen in 7000 ppm ZA1296 treated F2A litter, increased incidence of eye(s) shut was seen in 10ppm and 7000 ppm ZA1296 treated F2A litter. Increased age of preputial separation was observed in >10 ppm ZA 1296 treated F1 adults and in >350 ppm ZA1296 treated F2 adults. The slight delay in preputial separation did not result in any functional deficit in terms of reproductive performance. Increased eye opaqueness or cloudiness was seen in 7000 ppm ZA1296 treated F0 adult males, F1 adult males and females and F2A litter males at termination. Microscopic findings at termination showed increased eye unilateral or bilateral cataractous change in F0 adult male, F1 adult males and females and F2 litter males. Increased plasma tyrosine concentrations were seen in all treated groups in a dose dependent manner from F1 male and female adults and F2A male and female pups. Parental NOEL (No Observed Effect Level): <10 ppm due to reduced bodyweights and food consumption and increased plasma tyrosine level in F0 and F1 generations. Reproductive NOEL > 7000 ppm due to no effects in reproductive performance. Developmental NOEL: < 10 ppm due to reduced offspring bodyweights and food consumption and increased plasma tyrosine level in F1 and F2 generations. Acceptable. (Pan and Leung, 7/19/11)

53157-0027, 257959;* ZA1296 (Mesotrione) - Preliminary to multigeneration study in mice*; Rattray, N., 10/15/97. Central Toxicology Laboratory, Cheshire, UK SK 10 4TJ, Laboratory Project ID: Report Number: CTL/L/7897, Study Number: CTL/L/7897, Task Number: T007770-07, Supplement to Rec# 257957. Five groups of ten male and female Alpk: APfCD-1 mice were treated with 0, 10, 50, 350 and 7000 ppm ZA1296 in diet throughout the pre-mating, gestation and lactation periods, additional 5 groups of 5 mice/sex/group or more male and female mice were treated the same as satellite groups and were terminated two weeks after treatment. The fertility of the parental animals and clinical conditions, survival and growth of their offspring was measured. There was a significant decrease of percentage of pups born alive, litter size and pup survival in the 7000 ppm treated group. Body weight decrease was seen in the 7000 ppm group male pups on days 22 and 29, and there was slight body weight decrease (p>0.05) in the 7000 ppm group female pups at these time points. Total litter weights were significantly decreased at 7000 ppm ZA1296 throughout the study. Study Supplemental. (Pan and Leung, 7/21/11)

TERATOLOGY, RAT
** 53157-0022; 257942; "ZA1296: Developmental Toxicity Study in the Rat, First Revision"; (M.E. Moxon; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. RR0700; 6/28/99); Twenty four mated Wistar female rats/group were dosed orally by gavage with 0 (vehicle: deionized water), 100, 300 or 1000 mg/kg/day of ZA1296 (Mesotrione Technical) (batch no. P17; purity: 96.8%) from gestation day 7 through gestation day 16. No deaths resulted from the treatment. The mean body weight gain of the 1000 mg/kg dams was reduced after the first day of dosing. Thereafter, there was minimal effect upon the weight gain of the treated animals. The mean food consumption of the treated groups was reduced in a dose-related manner (p<0.05 or 0.01). The mean body weight of the 1000 mg/kg fetuses was less than the control value (p<0.01). The degree of fetal skeletal ossification was reduced in a dose-related manner in all of the treatment groups in comparison to the control group (p<0.05 or 0.01). No adverse effect evident. Maternal NOEL: <100 mg/kg/day (based upon the reduced food consumption noted for the dams in the100 mg/kg treatment group); Developmental NOEL: < 100 mg/kg/day (based upon the delayed ossification noted for the fetuses in the 100 mg/kg treatment group); Study acceptable. (Moore, 5/26/11)
TERATOLOGY, MOUSE
53157-0023 257944, 833; Teratology, mice; Central Toxicology Laboratory, Cheshire, UK SK10 4TJ, 6/29/99; Moxon, M.; Laboratory Project ID: Report #: CTL/P/6238, Study #: RM0793; ZA1296 (technical material), (batch P17, a light beige solid, 96.8% w/w purity) 10, 60, 150 or 600 mg/kg/day was administered to time-mated female Alpk:APfCD-1 mice (30 per group) orally from day 5 to 18 of gestation. In addition, 2 groups of mice (30 females per group) were dosed with water alone and provided the concurrent control data for the study. No treatment related effect on clinical condition, body weight gain or food consumption of the pregnant females or the number, growth, development or survival of the fetuses in utero was reported. Maternal NOEL = 600 mg/kg/day. Although there were transient, random and minor skeletal effects but there were no dose response relationship and therefore not considered to be treatment-related. Developmental NOEL = 600 mg/kg/day. 

Acceptable. (Pan and Leung, 5/31/11)

53157-0023, 257945;* ZA1296: Preliminary developmental toxicology study in the mouse *; Diggins, G., 9/5/97. Quintiles Toxicology/Pathology Services, Quintiles England Limited, Herefordshire HR8 1LH, UK, Laboratory Project ID: ICL/022/97, Supplement to Rec# 257944. 30 mg/ml or 60 mg/ml ZA1296 (beige liquid) was administered to mated female Crl:CD(ICR)BR mice (14 per group, for dose levels of 300 mg/kg/day or 600 mg/kg/day, respectively) orally from day 7 to 16 of gestation. In addition, a group of mice (14 mated females) were dosed with deionized water alone and provided the concurrent control data for the study. On day 19 of pregnancy, the females were killed and necropsy was performed. Maternal clinical signs, bodyweights and food consumption, numbers of corpora lutea, number and distribution of implantation sites were recorded. The fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities. No treatment related effect on clinical condition, body weight gain or food consumption of the pregnant females or the number, growth, development or survival of the fetuses in utero. Study Supplemental. (Pan and Leung, 6/1/11)

_53157-0024, 257953;" Prenatal Development Toxicity: Method Development Study in the Mouse to Achieve Compliance with EPA Guideline 870.3700-August 1998"; Moxon, M., 4/10/01. Central Toxicology Laboratory, Cheshire, UK SK10 4TJ, Laboratory Study ID: CTL Study #RM0811, Syngenta # 1254-98, Supplement to Rec# 257944. Fifty time-mated female mice (Alpk:AP;CD-1) were dosed by oral gavage with water on days 5 to 18 of gestation. The mice were terminated on day 19 of gestation. The dams were observed for clinical signs, body weights, food consumption, post mortem examination, number of corpora lutea and Gravid uterus weight. For the fetuses/litters, the following observations were made: number and position of implantations, number of live fetuses, number of intra-uterine deaths (early and late), fetal weight, sex, external and visceral variation and abnormality, skeletal variation and abnormality including evaluation of bone and cartilage and ossification of the manus and pes. The pregnancy rate was 74%. The mice that were delivered to the test facility on day 1, 2, or 3 of gestation had a decreasing pregnancy rate of 90.5%, 75.0% and 46.2%, respectively. Ten of the fifty mice on study did not survive to scheduled termination on day 19. Individual clinical observations and post mortem examinations suggested eight of the unscheduled deaths were due to poor dosing technique. There were no signs of ill health in mice surviving to scheduled termination. Major fetal defects were observed in eight fetuses from four litters including shortened bones in the fore and hindlimbs, cleft palate and no aortic arch. Study Supplemental. (Pan, 6/2/11)

TERATOLOGY, RABBIT
** 53157-0021; 257939; *ZA1296: Developmental Toxicity Study in the Rabbit, First Revision*; (M.E. Moxon; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. RB0684; 6/28/99); Twenty mated New Zealand White female rabbits/group were dosed orally by gavage with 0 (vehicle: deionized water), 100, 250 or 500 mg/kg/day of ZA1296 (Mesotrione Technical) (batch no. P17; purity: 96.8%) from gestation day 8 through gestation day 20. One doe
in the 100 mg/kg group died prior to initiation of dosing and one doe in the 250 mg/kg group was euthanized in extremis on day 22. One doe in the 100 mg/kg group and two each in the 250 and 500 mg/kg groups aborted after the termination of the treatment period and prior to the end of the study. There was no treatment-related effect on the maternal mean body weight gain or food consumption during the study. There were no developmental effects noted for the fetuses. **No adverse effect was evident.** Maternal NOEL: 100 mg/kg/day (based upon the incidence of abortions in the 250 mg/kg treatment group); Developmental NOEL: 500 mg/kg/day (based upon the lack of treatment-related effects in the 500 mg/kg treatment group); Study acceptable. (Moore, 5/25/11)

53157-0021; 257938; “ZA1296: Dose Range Finding Study in the Pregnant Rabbit”; (M.E. Moxon; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. RB0677; 12/11/97); Ten mated female New Zealand White rabbits/group were dosed orally by gavage with 0 (vehicle: deionized water), 450, 600 or 750 mg/kg/day of ZA1296 (batch no. P17, purity: 96.8%) from gestation day 8 through day 20. Five of the ten does in the 750 mg/kg group demonstrated severe weight loss by the conclusion of the dosing period. Three of the females in this group aborted. In the 600 mg/kg group, three of the does also lost weight, two of which subsequently aborted. The third one was euthanized for humane reasons. The ten animals in the 450 mg/kg group survived to the termination of the study. At scheduled termination, one doe in this group exhibited evidence of abortion in the uterus and another was found to have totally resorbed its litter. **No adverse effect indicated.** Study supplemental (non-guideline dose range-finding study). (Moore, 5/24/11)

0052, 258046; “Investigation of the Effects of ZA1296 and Tyrosine on Developmental Toxicity in the Rabbit” (Moxon, M.E., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, CTL Study Number RB0802, Syngenta Number 1281-99, 04/13/2000). Tyrosine (CTL test substance reference number; Y06512/001, free base minimum 98%), ZA1296 (technical material) (Batch reference number P17, purity = 96.8%), and/or water were administered to 20 time-mated female New Zealand White rabbits in various combinations per group as follows- group 1: water only by gavage, standard diet; group 2: 500 mg of ZA1296/kg/day by gavage, standard diet; group 3: 500 mg of ZA1296/kg/day by gavage, 1% tyrosine in standard diet; group 4: water only by gavage, 1% tyrosine in standard diet. Gavage doses were administered once daily on days 8-20 (inclusive) and diets containing 1% tyrosine were provided for the rabbits from the morning of day 8 until the morning of day 21. No treatment-related mortalities occurred. No treatment-related clinical signs were observed. Ophthalmoscopy revealed no treatment-related lesions. A treatment related decrease in body weight was observed in animals treated with 500 mg ZA1296/kg/day plus 1% tyrosine beginning on day 14; this body weight decrease was no longer statistically significant by day 24. Animals were bled prior to dosing on days 8, 14, and 20 and also 12 and 24 hours post dosing. Exposure to the mesotrione induced tyrosinemia in all 3 treatment groups 12 hours after the initiation of dosing (day 8), persisting throughout dosing interval (days 8 through 20), and decreasing but still persisting in the 500 mg ZA1296/kg/day only, and in the 500 mg ZA1296/kg/day plus 1% tyrosine dose groups at day 29. A treatment-related inhibition of kidney tyrosine aminotransferase activity was observed in the 500 mg ZA1296/kg/day only and in the 500 mg ZA1296/kg/day plus 1% tyrosine dose groups; a treatment-related inhibition in mean liver and mean kidney 4-hydroxyphenylpyruvate dioxygenase activity was observed in the 500 mg ZA1296/kg/day only and in the 500 mg ZA1296/kg/day plus 1% tyrosine dose groups. Macroscopic examination of the does revealed no treatment-related abnormalities. A decrease in the ratio of male fetuses to female fetuses was observed in the 500 mg ZA1296/kg/day only and in the 500 mg ZA1296/kg/day plus 1% tyrosine dose groups. Analyses of the number of litters, the mean fetal weight, the mean number of fetuses per animal, and the mean number of resorptions per animal revealed no treatment-related effects. Treatment-related extra vessels arising from aortic arch and cervical centra odontoid incompletely ossified were observed in the 500 mg ZA1296/kg/day only, and in the 500 mg ZA1296/kg/day plus 1% tyrosine dose groups.
Treatment-related incomplete ossification and no ossification of sternebra 5 and rib 13 long in length were observed in all 3 treatment groups. Pes and manus scores indicate treatment-related incomplete ossification in the 500 mg ZA1296/kg/day only and in the 500 mg ZA1296/kg/day plus 1% tyrosine dose groups. No adverse effects indicated. Maternal NOEL < 500 mg/kg/day (based on decreased maternal body weights and an inhibition in mean liver and mean kidney 4-hydroxyphenylpyruvate dioxygenase activity), Developmental NOEL < 500 mg/kg/day (based on incomplete ossification of various skeletal structures). Supplemental study (not a guideline study: only one dose level of the active ingredient was used and no dietary analysis on the test diets was performed). (Corlett and Leung, 11/09/2011)

GENE MUTATION

53157-0043 258012, “ZA 1296: An evaluation of mutagenic potential using S. Typhimurium and E.coli” 842; Zeneca Central Toxicology Laboratory, Cheshire, UK, Laboratory Project ID: Report #: CTL/P/4206, Study No: YV3205, 12/22/93; Callander, R. D. ZA 1296: CTL ref#: Y06684/004, Batch ref: P6, a light beige solid with purity of 98.1% w/w. ZA 1296 was tested in 4 strains of S. Typhimurium (TA 1535, TA1537, TA 98, and TA 100) and two strains of E.coli (WP2P and WP2P uvrA) for its ability to cause reverse mutation with or without metabolic activation. Six concentrations of the test substance were tested: 5000, 2500, 1000, 500, 200 and 100 ug/plate. In the phase 1 and 2 of the study, plate-incorporation method was used for the 6 strains under metabolic activation and no activation, in phase 3, plate-incorporation method was used for the tests done without metabolic activation and pre-incubation method was used for tests done with metabolic activation. The test substance did not induce significant increase of revertant colony numbers at the 6 concentrations tested under metabolic activation or no activation except for a few incidental changes. Positive control materials induced expected increases in mean revertant colony number per plate over that of the negative controls. Study acceptable. (Pan and Leung, 8/18/11).

53157-0043 258010, “ZA 1296: An evaluation of mutagenic potential using L5178Y mouse lymphoma cells” 842; Zeneca Central Toxicology Laboratory, Cheshire, UK, Laboratory Project ID: Report #: CTL/P/4183, Study No: VV0098, 2/28/94; Clay, P., ZA 1296: ref#: R251296, Batch ref: P6, CTL ref.#: Y06684/004, a light beige solid with purity of 98.1% w/w. ZA 1296 (1000, 500, 250 and 125 ug/ml) was tested in L5178Y mouse lymphoma cells in the absence and presence of S9 mix for its mutational potential. The treatment lasted for 4 hours followed by 72 hours of phenotypic expression in fresh medium. The mutant cells were selected in the selective medium for additional 10-12 days with TFT (trifluorothymidine, 4ug/ml). The mutation frequency was determined by comparing the plating efficiencies of cultures in selective medium with those of cultures in non-selective medium. The experiment was repeated. There were no significant changes of cell survival or mutation frequency in the test material treated cultures compared with those treated with solvent control, DMSO. Positive control materials induced expected increases in mean revertant colony number per plate over that of the negative controls. Study acceptable. (Pan and Leung, 9/2/11).

CHROMOSOME EFFECTS

53157-0043 258008, “ZA 1296: An evaluation in the in vitro cytogenetic assay in human lymphocytes” 842; Zeneca Central Toxicology Laboratory, Cheshire, UK, Laboratory Project ID: Report #: CTL/P/4187, Study No: SV0700, 2/21/94; Griffiths, K and Mackay, J., ZA 1296: CTL ref#: Y06684/004, Batch ref: P6, a light beige solid with purity of 98.1% w/w. ZA 1296 (from 10 or 250 to up to 2000 ug/ml) was tested for its clastogenic potential in an in vitro cytogenetic assay in human lymphocytes. The tests were done using lymphocytes from two donors (male and female) in the presence and absence of metabolic activation system (S9-mix). Statistically significantly increased mean percentage of aberrant cells excluding gaps was noted in 1500 and 1000 ug/ml ZA 1296 treated lymphocytes from donor 1 without metabolic activation at sampling time of 68 hours. Positive control materials induced expected increases of percentages of aberrant cells in both donors in the presence or absence of the metabolic activation system. There was no significant
change of mean percentage of aberrant cells over untreated or solvent control values in the absence of metabolic activation at 68 hours for the female donor, in the presence of metabolic activation in both donors at 68 hours as well as in the presence or absence of metabolic activation in the female donor at 92 hours. Study acceptable. (Pan and Leung, 8/26/11).

DNA DAMAGE

53157-0042 258002, “ZA1296: An evaluation in the mouse micronucleus test” 844; mice Zeneca Central Toxicology Laboratory, Cheshire, UK, Laboratory Project ID: CTL/P/4249, 2/11/94; Griffiths, K. and Mackay, J. M., ZA1296: sponsor ref.#: Al/93/0011, CTL ref#: Y06684/004, Batch ref: P6, a light beige solid with purity of 98.1% w/w. Phase I of the study was conducted to determine the maximum tolerated dose (MTD): Groups of 2 male mice were given a single oral dose of 320 or 2000 mg/kg ZA1296 and groups of 5 male and 5 female mice were given a single oral dose of 500 or 800 mg/kg ZA1296. Based on lethality or severe toxicity observed over four to five days, 500 mg/kg was chosen as the MTD for the main study. In the Phase II of the study 5 male and 5 female mice were given a single oral dose of double deionised water, 500 mg/kg ZA1296 or 65 mg/kg cyclophosphamide (positive control), and bone marrow was extracted 24 or 48 hours later for vehicle control and ZA1296 treated groups, or 24 hours later for positive control group. There was a small but statistically significant increase of mean micronucleated polychromatic erythrocytes/1000 polychromatic erythrocytes in the bone marrow of ZA1296 treated females at 48 hours after exposure over that of the control females. Extended counts of mean micronucleated polychromatic erythrocytes in the bone marrow of the same animals at 48 hours showed no statistically significant change between the 500 mg/kg ZA1296 treated females and control females. The positive control induced expected marked increase of the mean micronucleated polychromatic erythrocytes in the bone marrow over those of the control mice. The mean percentages of polychromatic erythrocytes in 1000 erythrocytes counted stayed the same for different treatment groups. The results of the study indicated the ZA1296 was unlikely to be a clastogenic agent. Study acceptable. (Pan and Leung, 8/8/11).

NEUROTOXICITY

Rat Acute Neurotoxicity

0050, 258042; “ZA1296: Acute Oral Toxicity to the Rats” (Horner, S.A., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Laboratory Project ID: Report No. CTL/P/5631 Study No. AR6196, 10/30/1997). 870.62. ZA1296 (Batch reference number P22, purity = 97.5%), prepared in deionized water, was administered in a single dose by gavage to 10 Alpk:AP;SD rats per sex per dose at dose levels of 0 (deionized water only), 20, 200, and 2000 mg/kg. No treatment-related mortalities occurred. No treatment-related clinical signs were observed. No effects on body weight were observed. FOB assessments revealed no treatment-related effects on days 1, 8, and 15. Motor activity assessments revealed no treatment-related effects on days 1, 8, and 15. Macroscopic examination revealed no treatment-related abnormalities. No adverse effects. NOEL (M/F) = 2000 mg/kg (based on no effects at the highest dose tested). Acceptable. (Corlett and Leung, 7/11/2011)

Rat Subchronic Neurotoxicity

0053; 258048; “ZA1296: Subchronic Neurotoxicity Study in Rats” (Horner, S.A., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Laboratory Project ID: Report No. CTL/P/5630 Study No. PR1043, 10/31/1997). 870.6200. ZA1296 (Batch reference number P22, purity = 97.6%) was admixed to the diet and fed to 12 Alpk:AP;SD rats per sex per dose at dose levels of 0 (diet only), 2.5, 100, or 5000 ppm (0, 0.20, 8.25, and 402.80 mg/kg/day, respectively for males, and 0, 0.23, 9.29, and 466.64 mg/kg/day, respectively for females) continuously for 90 consecutive days. No treatment-related mortalities occurred. Treatment-related eye opacities were observed 12 times in 3 males at 100 ppm between weeks 9
and 14, 54 times in 10 males at 5000 ppm between weeks 8 and 14, and 15 times in 4 females at 5000 ppm between weeks 10 and 14. A treatment-related decrease (statistically significant in females) in mean body weight (adjusted for initial weight) when compared to the control group was observed in both sexes at 5000 ppm. FOB and motor activity assessments revealed no treatment-related effects. No treatment-related effects on brain weight, width, or length were observed. Week 13 ophthalmoscopic examinations revealed treatment-related corneal opacity and/or hazy cornea with or without corneal vascularization in males at 100 and 5000 ppm and in females at 5000 ppm. Macroscopic examination revealed treatment-related opaque eyes in males at 100 and 5000 ppm and in females at 5000 ppm. Neuropathological examination of the central and peripheral nervous system tissue revealed no treatment-related abnormalities. Possible adverse effect: Ocular toxicity: opacities were observed both clinically and at post mortem examination in males and females. NOEL (M) = 0.20 mg/kg/day (2.5 ppm) and NOEL (F) = 9.29 mg/kg/day (100 ppm), both based on ocular toxicity. Acceptable. (Corlett and Leung, 07/19/2011)

Preliminary Mouse Dose Range-Finding for Developmental Neurotoxicity
0044; 258015; “Mesotrione: Summary of Data from a Preliminary Exposure Study and Recommendations for Dose Selection in the Developmental Neurotoxicity (DNT) Study in the Mouse” (Akins, J. et al., Human Safety Assessment, Syngenta Crop Protection, Inc., Syngenta Central Toxicology Laboratory, Cheshire, UK, Laboratory Study Identification: Syngenta Number T004222-05, 05/13/2005). Mesotrione (no lot number reported, no purity information provided) was administered orally in the diet to 12 mice (strain not reported) at dose levels of 0 (control) 10, 100, 1000, or 7000 ppm (0, 1.6, 15.8, 154.5, and 1076.9 mg/kg/day for parent females during gestation days 6 to 19 and 0, 3.5, 37.0, 359.8, and 2238.4 mg/kg/day for parental females during days 1 to 23 post partum). F0 and F1 body weights and food consumption were recorded throughout the study interval. Five dams and fetuses/pups per dose level were sacrificed on day 19 of gestation, and on days 5, 12, 18, and 23 post partum. Terminal blood samples were collected from each animal, plasma was pooled by sex and litter, and the plasma analyzed for tyrosine and mesotrione. No treatment-related effects on mean maternal body weight or mean maternal food consumption were observed during gestation. A treatment-related decrease maternal body weight at 7000 ppm was observed during the post partum interval. No treatment-related effects on the duration of gestation, % of live born pups, litter size, pup survival, or pup sex distribution were observed. A treatment-related decrease in mean pup body weight at 7000 ppm was observed beginning in males at day 8 and in females at day 5. Dose-related increases in plasma mesotrione levels were observed in all mesotrione exposed dams, day 19 gestation fetuses, and pups during lactation. A statistically significant increase in plasma tyrosine levels when compared to the control values was observed in dams, fetuses (day 19 gestation), and pups at all time points at dose levels of 10 ppm and higher in a dose-related manner. No adverse effects. NOEL not determined. Summary study. (Corlett and Leung, 7/25/2011)

RAT METABOLISM
53157-0029, 257965;” ZA1296: Systemic exposure following dietary administration “; Hall, M G., 6/10/96. Central Toxicology Laboratory, Cheshire, UK, Laboratory Project ID: CTL/L/7179. Groups of 18 male Alpk:APfSD (wistar-derived) rats were fed diets containing 1, 40, 125, 1250, and 5000 ppm ZA1296, from batch P8 (purity 93.3%) or P11 (purity 95.1%) for 7 days. ZA1296 was measured in plasma and urine, plasma tyrosine level and food consumption was also measured. Total food consumed during the study showed no difference between the groups fed with 2 batches of ZA1296. The percentage of unchanged ZA1296 excreted in the urine during the final 48 hours showed dose-related increase in the batch P8 fed groups, there was no inter-batch differences ranging from 40 to 5000 ppm test compound. The area under the plasma concentration of ZA1296 versus time curve (AUC) during the final 24 hours of the feeding period showed a slight difference between the batches, the linear increase in AUC with increasing dose was observed in both batches. The high dose levels of batch P11 results in slightly higher systemic exposure than P8 measured by both AUC and maximum plasma concentrations of ZA1296. Plasma tyrosine level in
the first 24 hours after dosing showed no difference between dietary treatments of two batches of test compound at 1 ppm or higher. **Study Supplemental.** (Pan, 8/1/11)

53157-0046; 258025; "ZA1296: Excretion and Tissue Retention of a Single Oral Dose (100 mg/kg) in the Rat"; (D. Macpherson; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. UR0501; 5/17/96); Five Wistar-derived rats/sex were dosed orally by gavage with 100 mg/kg of [14C]-aromatic ring-labeled ZA1296 (reference no. Y06684/159, specific activity: 1.42 GBq/mmol; radiochemical purity: 98.1%). Unlabeled ZA1296 (reference no. Y06684/008, purity: 99.3%) was used to adjust the specific activity of the dosing preparation. Urine was the predominant site of radiolabel recovery with 61 to 63% of the administered dose being present in the urine by 72 hours post-dose. Approximately 30% of the dose was recovered in the feces over the same time period. Approximately 85% of the administered dose was excreted within 24 hours post-dose. Approximately 1% of the administered dose was sequestered in the tissues, primarily the liver and kidneys, at 72 hours post-dose. **Supplemental study.** (Moore, 7/1/11)

53157-0046; 258026; "ZA1296: Biotransformation in the Rat"; (A. Gledhill; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. UR0442; 6/3/96); Two Wistar-derived rats/sex/group were dosed orally with 50 mg/kg of [14C-dione]-ZA1296 (reference no. Y06684/010, specific activity: 1.35 GBq/mmol; radiochemical purity: 99.5%) or 50 or 100 mg/kg of [14C-aromatic]-ZA1296 (reference no. Y06684/011, specific activity: 1.12 GBq/mmol; radiochemical purity: 100%). Unlabeled ZA1296 (reference no. Y06684/008, purity: 99.3%, reference no. Y06684/005, purity: 99.5%) was used to adjust the specific activity of the dosing preparations. The study animals in the two 50 mg/kg treatment groups had their bile ducts cannulated. Urine, feces and bile were collected up to 48 hours post-dose. The urine, fecal and bile samples were pooled according to sex and metabolites were identified and quantified in the respective samples by HPLC-MS and Proton Nuclear Magnetic Resonance Spectroscopy (1H-NMR). In addition, urine and fecal samples recovered from three other studies (vol. no. 53157-0046, rec. nos. 258025, 258027 and 258029) were analyzed for metabolites. In the profile of the two 50 mg/kg groups, excretion was predominantly via the urine with 59 to 76% of the recovered radiolabel in that fraction. A slightly greater portion of the excreted radiolabel was recovered in the bile of the males than in the bile of the females (10 to 14% of the administered dose vs. 2 to 4%). Absorption of the test material constituted 51 to 66% of the administered dose. The unmetabolized parent compound comprised between 63.7 and 79% and 74.7 and 87.0% of the recovered radiolabel for the males and females, respectively, and was recovered almost entirely in the urine. Two metabolites, AMBA and MNBA, resulting from the cleavage of the ketone bridge were recovered predominantly in the feces. Among the metabolites which were recovered and identified, hydroxylation of the dione ring was noted. Other moieties which were not identified constituted up to 6% of the administered dose. **Study supplemental.** (Moore, 7/6/11)

53157-0046; 258027; "ZA1296: Excretion and Tissue Retention of a Single Oral Dose (1 mg/kg) in the Rat"; (D. Macpherson; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. UR0502; 5/20/96); Five Wistar-derived rats/sex were dosed orally by gavage with 1.0 mg/kg of [14C]-aromatic ring-labeled ZA1296 (reference no. Y06684/159, specific activity: 1.42 GBq/mmol; radiochemical purity: 98.1%). Urine was the predominant site of radiolabel recovery with 54 to 56% of the administered dose recovered in the urine by 72 hours post-dose. Approximately 25% of the administered dose was in the feces over the same time period. Seventy four percent of the administered dose was excreted within 24 hours post-dose. Approximately 13% of the administered dose was sequestered in the tissues, primarily the liver and kidneys, at 72 hours post-dose. **Supplemental study.** (Moore, 7/5/11)
ZA1296: Excretion and Tissue Retention of a Single Intravenous Dose (1 mg/kg) in the Rat; (D. Macpherson; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. UR0522; 5/29/96); Five Wistar-derived rats/sex were dosed intravenously via the tail vein with 1.0 mg/kg of [14C-aromatic]-ZA1296 (reference no. Y06684/159, specific activity: 1.42 GBq/mmol; radiochemical purity: 99.4%). Urine was the predominant site of radiolabel recovery with 79 to 84% of the administered dose being present in the urine by 72 hours post-dose. Two to 7% of the dose was recovered in the feces over the same time period. Eighty three to 84% of the administered dose was excreted within 24 hours post-dose. Approximately 10% of the administered dose was sequestered in the tissues, primarily the liver and kidneys, at 72 hours post-dose. **Supplemental study.** (Moore, 7/6/11)

ZA1296: Excretion and Tissue Retention of a Single Oral Dose (1 mg/kg) in the Rat Following Repeat Dosing; (D. Macpherson; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. UR0525; 5/24/96); Eight Wistar-derived rats/sex were dosed orally with 1.0 mg/kg/day of unlabeled ZA1296 (reference no. Y06684/008, purity: 99.3%) for 14 days. On the 15th day, five Sprague-Dawley rats/sex were dosed orally by gavage with 1.0 mg/kg of [14C]-aromatic ring-labeled ZA1296 (reference no. Y06684/159, specific activity: 1.42 GBq/mmol; radiochemical purity: 98.1%). Urine was the predominant site of radiolabel recovery with 60 to 67% of the administered dose being present in the urine by 72 hours post-final dose. Twenty three to 30% of the dose was recovered in the feces over the same time period. Eighty five to 87% of the administered dose was excreted within 24 hours post-final dose. Approximately 5% of the administered dose was sequestered in the tissues, primarily the liver and kidneys, at 72 hours post-final dose. **Supplemental study.** (Moore, 7/5/11)

ZA1296: Excretion, Tissue Distribution and Metabolism of a Single Oral Dose (1 mg/kg and 100 mg/kg) in the Mouse; (A.J. Gledhill; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. UM0573; 11/13/97); Four CD-1 mice/sex/group were dosed orally with 1.0 or 100 mg/kg of [14C]-aromatic ring-labeled ZA1296 (reference no. Y06684/218, specific activity: 1.42 GBq/mmol; radiochemical purity: 97%). Unlabeled ZA1296 (reference no. Y06684/008, purity: 99.3%) was used to adjust the specific activity of the 100 mg/kg treatment preparation. Urine was the predominant site of radiolabel recovery with 40 to 58% of the administered dose being present in the urine of the 1.0 mg/kg treatment group by 72 hours post-dose. The percent of the administered dose recovered in the urine increased to 63 to 70% for the 100 mg/kg treatment group. Twenty one to 38% and 24 to 27% of the dose was recovered in the feces of the 1.0 and 100 mg/kg treatment groups, respectively, over the same time period. Sixty seven to 76% of the administered dose was excreted within 24 hours post-dose for the 1.0 mg/kg group. For the 100 mg/kg group, 83 to 91% of the dose was excreted in the first 24 hours post-dose. Fourteen to 15% of the administered dose was still sequestered in the tissues, primarily the liver and kidneys, of the 1.0 mg/kg group at 72 hours post-dose. In the 100 mg/kg group, the percent of the administered dose still in the tissues at 72 hours had declined to less than 1%. The unmetabolized parent compound comprised between 49 and 65% of the administered dose for the 1.0 mg/kg group and 70 to 78% of the dose for the 100 mg/kg group. It was primarily recovered in the urine. Two metabolites, AMBA and MNBA, resulting from the cleavage of the ketone bridge were recovered predominantly in the feces. **Supplemental study.** (Moore, 7/7/11)

ZA1296: Whole Body Autoradiography Study in the Rat Following a Single Oral Dose (Mg/Kg); (E. Prescott, D. Bennett; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. PR0990; 9/19/95); One Wistar-derived rat/sex/group was dosed with 5 mg/kg of [14C-dione] ZA1296 (specific activity: 1.35 GBq/mmol, radiochemical purity:
>99%) or [\(^{14}\text{C-}	ext{aromatic}\)] ZA1296 (specific activity: 1.12 GBq/mm, radiochemical purity: >97%) and euthanized at 24 hours post-dose. A second cohort of one rat/sex/group were dosed with 5 mg/kg of either test material and euthanized at 48 hours post-dose. Urine, fecal and/or carbon dioxide and other volatiles were recovered periodically until the animals were euthanized. Whole body autoradiographs of each animal were prepared. Excretion of the radiolabel in the urine constituted from 60 to 72% (urine + cage wash) of the administered dose by 48 hours. The percent of the administered dose which was recovered in the feces ranged from 14 to 30% over the same time period. Approximately 0 to 1% of the administered dose was recovered as a volatile entity and carbon dioxide when the animals were treated with [\(^{14}\text{C-dione}\)] ZA1296. In the whole body autoradiographs, the predominant sites of labeling were in the kidneys, liver and gastrointestinal tract. **Supplemental study.** (Moore, 6/30/11)

**SUBCHRONIC STUDIES**

**Rat 28-Day Dietary Toxicity Study**

0008, 257901; “ZA1296: 28 Day Toxicity Study in the Rat” (Milburn, G.M., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Report No. CTL/R/1308, Study No. XR6167, 11/18/1997). 870.3050. Eight batches 1 kilogram of diet were prepared, four by admixing ZA1296 (Batch P17 (WRC 15213-17-1), purity = 96.8%) to the diet at a concentration of 100 ppm and then adding 0 g, 5 g, 10 g, or 25 g of tyrosine per diet. Four additional diets were prepared by adding 0 g, 5 g, 10 g, or 25 g of tyrosine to each of the four batches of control diet. 8 groups of 8 female Alpk:APfSD rats were each fed one of the above diets for 28 days consecutive days. Cloudy eyes were observed in the animals treated with the test article with added tyrosine (0.5% to 2.5%) from weeks 3 to 5. A treatment related decrease in body weight and food consumption was observed at the 100 ppm of test article plus 2.5% tyrosine dose group. Ophthalmoscopy findings prior to termination revealed corneal opacity in all animals dosed with 100 ppm of the test article plus added tyrosine and vascularization in animals dosed with 100 ppm of test article plus 1.0% and 2.5% tyrosine. Mean plasma tyrosine levels were elevated with the addition of tyrosine into the diet and increased even further when 100 ppm of the test article and tyrosine were added to the diet; the extent of the increase was dependent on the amount of tyrosine added into the diet. A statistically significant increase in mean tyrosine aminotransferase activity was observed in animals treated with 100 ppm of the test article (with and without added tyrosine) and in animals treated with control diet with 2.5% tyrosine added; a statistically significant decrease in p-hydroxyphenylpyruvate dioxygenase activity was observed at these same dose levels. A statistically significant increase in mean relative liver weight was observed in animals treated with 100 ppm of the test article (with and without added tyrosine) and in mean relative kidney weight in the animals treated with 100 ppm of the test article with 2.5% tyrosine added. Macroscopic examination revealed cloudy eyes in all animals treated with 100 ppm of the test article plus added tyrosine (0.5, 1.0, and 2.5%). **Possible adverse effect indicated: corneal opacity.** NOEL not determined. **Supplemental study** (not a guideline study, only females were used, and no analyses of the dosing materials used were submitted). (Corlett and Leung, 08/02/2011)

0019, 257934; “Mesotrione: Dynamic Exposure (28 Day Duration in the Rat)” (Lees, D., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, CTL Study Number XR6680, Syngenta Number 1632-00, 08/31/2000). Mesotrione technical (Batch reference number P22, purity = 97.8%) was admixed to the diet and fed to a main group animals consisting of 20 male Alpk:APfSD (Wistar-derived) rats per dose at dose levels of 0 (untreated diet) and a set of variable dose levels (100 ppm (days 1 and 2), 30 ppm (days 3 and 4), 10 ppm (days 5 and 6), 3 ppm (days 7 and 8), 1 ppm (days 9 and 10), 0.3 ppm (days 11-14), 100 ppm (days 15 and 16), 30 ppm (days 17 and 18), 10 ppm (days 19 and 20), 3 ppm (days 21 and 22), 1 ppm (days 23 and 24), 0.3 ppm (days 25-28) for 28 consecutive days, and to a satellite group of 8 control male rats and 44 treated rats that were treated the same as the main group animals but sacrificed throughout the treatment period as follows: 4 treated animals and 4 control animals on day 3, 4 treated animals on day 5, 4 treated
animals on day 7, 4 treated animals on day 11, 4 treated and 4 control animals on day 15, 4 treated animals on day 17, 4 treated animals on day 19, 4 treated animals on day 21, 4 treated animals on day 23, and 4 treated animals on day 25. The overall mean value of the dose of mesotrione received was 2.39 mg/kg/day with a range of 0.025-13.03 mg/kg/day. No mortalities occurred. No treatment-related clinical signs were observed. Ophthalmoscopy revealed no treatment-related lesions. A treatment related decrease in body weight was observed in the treated animals. Exposure to the mesotrione induced tyrosinemia with plasma tyrosine levels related to the administered mesotrione levels. A treatment-related induction of mean liver tyrosine aminotransferase activity was observed; a treatment-related inhibition in mean liver 4-hydroxyphenylpyruvate dioxygenase activity was observed at all dose levels. Macroscopic examination revealed no treatment-related abnormalities. **No adverse effects indicated.** NOEL (M) < 0.3 ppm based on inhibition of liver 4-hydroxyphenylpyruvate dioxygenase activity.

**Supplemental study** (not a guideline study: only male animals were used, no organ weights were determined, and no histopathology was performed). (Corlett and Leung, 11/01/2011)

Rat 90-Day Dietary Toxicity Studies

0010; 257905; “ZA1296: 90 Day Dietary Study in Rats” (Horner, S.A., Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Laboratory Project ID: Report No. CTL/P/4456, Study No. PR0972, 02/08/1995). 870.3100. ZA1296 (Batch P8, purity = 93.3%) was admixed to the diet and fed to 12 Alpk:APFSD rats per sex per dose at dose levels of 0 (diet only), 1, 125, 1250, or 12500 ppm (0, 0.09, 11, 112, and 1111 mg/kg/day, respectively for males, and 0, 0.1,13, 126, and 1213 mg/kg/day, respectively for females) for 90 days. No mortalities occurred. Treatment-related opaque eyes were observed in males at 125, 1250 and 12500 ppm and in females at 1250 and 12500 ppm, and tray papers stained purple and/or yellow were observed in males at 12500 ppm and in females 1250 and 12500 ppm. A treatment-related decrease in mean body weight (adjusted for initial weight) when compared to the control group was observed in both sexes at 125, 1250, and 12500 ppm. A treatment-related decrease in mean food consumption was observed in both sexes at 12500 ppm. Ophthalmoscopic findings included treatment-related corneal opacity or hazy opacity, and corneal vascularization in males at 125, 1250, and 12500 ppm and in females at 1250 and 12500 ppm. A treatment-related decrease in the mean red blood cell level was observed in both sexes at 12500 ppm and treatment-related increases in mean cell volume and mean cell hemoglobin levels were observed in males at 12500 ppm. Treatment-related increases in the mean plasma creatinine level in both sexes at 125, 1250, and 12500 ppm and in the mean triglycerides level in females at 125, 1250, and 12500 ppm were observed. Urine clinical chemistry revealed a treatment-related increase in the mean protein level in females at 12500 ppm. A dose-related increase in liver weight adjusted to body weight (determined by analysis of covariance) was observed in both sexes at 125, 1250, and 12500 ppm and a dose-related increase in kidney weight adjusted to body weight (determined by analysis of covariance) was observed in males at 125, 1250, and 12500 ppm. Macroscopic examination revealed treatment-related opaque eyes in males at 125, 1250, and 12500 ppm and in females at 12500 ppm. Microscopic examination revealed treatment-related keratitis with or without vascularization of the cornea in males at 125, 1250, and 12500 ppm and in females at 1250 and 12500 ppm. **Possible adverse effect:** Ocular toxicity: opacities were observed during clinical observations, during ophthalmoscopic examination, and at post mortem examination in males and females. NOEL (M) = 0.09 mg/kg/day (1 ppm) and NOEL (F) = 0.1 mg/kg/day (1 ppm), based on decreased mean body weight and ocular toxicity. **Acceptable.** (Corlett and Leung, 08/29/2011)

0011; 257907; “ZA1296: 90 Day Dietary Toxicity Study in Rats” (Pinto, P.J., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Laboratory Project ID: Report No. CTL/P/4986, Study No. PR1027, 11/05/1997). 870.3100. ZA1296 (technical) (Batch reference number P17 (WTC 15213-17-1), purity = 96.8%) was admixed to the diet and fed to 12 Alpk:APFSD rats per sex per dose at dose levels of 0 (diet only), 2.5, 5.0, 7.5, or 150 ppm (0, 0.21, 0.41, 0.63, and 12.46 mg/kg/day, respectively for males, and 0, 0.23, 0.47, 0.71, and 14.48 mg/kg/day, respectively for females) for 90 consecutive days. No mortalities occurred. No
toxicologically-related effect on body weight was observed. Ophthalmoscopic findings at termination included treatment-related corneal opacity or hazy opacity, and corneal vascularization in males at 7.5 and 150 ppm and corneal opacity in females at 150 ppm. Statistically significant increases in mean hematocrit and mean cell volume levels were observed in females at 150 ppm. Qualitative tests on the urine revealed an increase in protein and ketones in males at 7.5 and 150 ppm and an increase in ketones in females at 150 ppm. No treatment-related effects on the organ to body weight ratios were observed. Macroscopic examination revealed treatment-related opaque eyes in males at 7.5 and 150 ppm. Microscopic examination revealed eyes with treatment-related keratitis in males at 7.5 and 150 ppm and in females at 150 ppm. Possible adverse effect: Ocular toxicity: corneal opacity was observed during clinical observations and at the terminal ophthalmoscopic examination. NOEL (M) = 0.41 mg/kg/day (5.0 ppm) and NOEL (F) = 0.71 mg/kg/day (7.5 ppm), based on corneal opacity. Acceptable. (Corlett and Leung, 09/07/2011)

0013, 257911; “ZA1296: 90 Day Dietary Study in Rats to Investigate Selective Non-Ocular Toxicity End Points” (Brammer, A., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Laboratory Project ID: Report No. CTL/T/2887, Study No. PR0990, 07/24/1995). ZA1296 Technical (Batch P11, purity = 95.1%) was admixed to the diet and fed to 12 Alpk:APfSD male rats per dose at dose levels of 0 (untreated diet), 10, 20, 50, or 125 ppm  (0, 0.9, 1.7, 4.3, and 10.7 mg/kg/day, respectively) for 90 days. No treatment-related mortalities occurred. Treatment-related opaque eyes were observed at all dose levels. A treatment-related slight decrease in adjusted mean body weight was observed at 125 ppm. Ophthalmoscopic findings included treatment-related corneal opacity or hazy opacity, and corneal vascularization at all dose levels. Possible adverse effect: ocular toxicity; corneal opacity was observed during clinical observations and at the terminal ophthalmoscopic examination. No dose-related effects on liver and kidneys weights were observed. NOEL (M) < 0.9 mg/kg/day (10 ppm) (corneal opacity). Supplemental study (not a guideline study: only male test animals were used, no hematology and no clinical chemistry were conducted on serum of the test animals, and no macroscopic and no microscopic examinations of the internal organs of the test animals were performed). (Corlett and Leung, 09/29/2011)

0015, 257915; “ZA1296: 90 Day Dose Response Study in Male Rats” (Brammer, A., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Report No. CTL/R/1304, Study No. XR5997, 11/19/1997). ZA1296 (Batch reference number P17, purity = 96.8%) was admixed to the diet and fed to 16 male Alpk:APfSD rats per dose at dose levels of 0 (untreated diet), 0.5, 1, 3, 4, 5, 7.5, 10, or 100 ppm (0, 0.04, 0.09, 0.27, 0.35, 0.44, 0.67, 0.89, and 8.96 mg/kg/day, respectively) for 90 consecutive days. No treatment-related mortalities occurred. Treatment-related cloudy eyes were observed at dose levels of 5 ppm and above. Pre-terminal ophthalmoscopic examination revealed treatment-related hazy corneal opacity and/or corneal vascularization at dose levels of 7.5 ppm and above. A statistically significant and dose-related increase in mean plasma tyrosine levels was observed at dose levels of 1 ppm and above 24 hours after the commencement of dosing and at all dose levels at week 1 and week 14. NMR analyses of urinary phenolic acids revealed a dose-related increase in both conjugated and free phenolic acids with the proportion of conjugated to free phenolic acids becoming less at the higher dose levels. A dose-related increase in liver weight adjusted to final body weight (determined by analysis of covariance) was observed at dose levels of 4 ppm and above and a dose-related increase in kidney weight adjusted to final body weight was observed at dose levels of 5 ppm and above. Other than the eye, no treatment-related abnormalities were observed during macroscopic examination. Microscopic examination and electron microscopy of liver and kidney tissues revealed no treatment-related abnormalities. Treatment-related induction of liver tyrosine aminotransferase (TAT) activity was observed at week 14 at dose levels of 3 ppm and above and treatment-related inhibition of liver 4-hydroxyphenylpyruvate dioxygenase (HPPD) activity was observed at week 14 at all dose levels. Possible adverse effect indicated- Ocular toxicity: corneal opacity was observed during clinical observations and at the terminal
ophthalmoscopic examination. NOEL (M) < 0.04 mg/kg/day (0.5 ppm) based on increased mean plasma tyrosine levels. **Supplemental study** (not a guideline study: only males were used, no hematology and no clinical chemistry analyses were conducted on the blood of the test animals, and microscopic examinations were conducted on liver and kidney tissues only). (Corlett and Leung, 10/13/2011)

0015, 257916; “ZA1296: 90 Day Dose Response Study in Female Rats” (Brammer, A., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Report No. CTL/R/1315, Study No. XR6195, 11/19/1997). ZA1296 (Batch reference number P17, purity = 96.8%) was admixed to the diet and fed to 12 female Alpk:AP;SD rats per dose (with an additional 8 rats per dose as satellites) at dose levels of 0 (untreated diet), 1, 5, 10, 100, 1000, or 2500 ppm (0, 0.09, 0.48, 0.95, 4.82, 9.54, 94.83, and 236.75 mg/kg/day, respectively) for 90 consecutive days (satellite animals were sacrificed on days 8 or 29). No treatment-related mortalities occurred. Treatment-related cloudy eyes were observed at 1000 and 2500 ppm. Pre-terminal ophthalmoscopic examination revealed treatment-related hazy corneal opacity and/or corneal opacity with or without corneal vascularization at 1000 and 2500 ppm. A treatment-related decrease in mean body weight (adjusted for initial weight) when compared to the control group and a decrease in mean food consumption were observed at 2500 ppm. A statistically significant and dose-related increase in mean plasma tyrosine levels was observed at dose levels of 5 ppm and above during weeks 2, 5, and 14. Commencing at 100 ppm, NMR analyses of urinary phenolic acids revealed a dose-related increase in both conjugated and free phenolic acids with the proportion of conjugated to free phenolic acids becoming less at the higher dose levels during week 5. A dose-related increase in liver weight adjusted to final body weight was observed at dose levels of 50 ppm and above in animals sacrificed at week 14; no treatment-related effect on kidney weight was observed. Treatment-related induction of liver tyrosine aminotransferase (TAT) activity was observed at week 2 at dose levels of 5 ppm and above and at weeks 5 and 14 at dose levels of 1000 and 2500 ppm; treatment-related inhibition of liver 4-hydroxyphenylpyruvate dioxygenase (HPPD) activity was observed at weeks 2, 5 and 14 at all dose levels. **Possible adverse effect indicated—ocular toxicity:** corneal opacity was observed during clinical observations and at the terminal ophthalmoscopic examination. NOEL (F) < 0.09 mg/kg/day (1 ppm) based on inhibition of liver 4-hydroxyphenylpyruvate dioxygenase (HPPD) activity. **Supplemental study** (not a guideline study: only females were used, no hematology and no clinical chemistry analyses were conducted on the blood of the test animals, and no macroscopic and no microscopic examinations were conducted on internal organs). (Corlett and Leung, 10/18/2011)

0016, 257924; “ZA1296: 90 Day Reversibility Studies in Rats” (Brammer, A. and Provan, W.M., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Report No. CTL/R/1302, Study Nos. PR1066 & XR6242, 11/19/1997). ZA1296 (Batch reference number Batch P17, purity = 96.8%) was admixed to the diet and fed to 2 sets of male Alpk:AP;SD rats each of which consisted of 40 rats per dose level; one of sets consisted of dose levels of 0 and 2500 ppm (0 and 192 mg/kg/day, respectively) and the other set consisted of dose levels of 0, 5, and 100 ppm (0, 0.37, and 7.52 mg/kg/day, respectively). Rats were treated for 90 consecutive days. For the first set of rats, 8 rats per group were sacrificed at the following time points: after 90 days of treatment, after 1 week of recovery, after 2 weeks of recovery, after 4 weeks of recovery, and after 9 weeks of recovery; for the second set of rats, 8 rats per group were sacrificed at the following time points: after 90 days of treatment, after 2 weeks of recovery, after 4 weeks of recovery, after 6 weeks of recovery, and after 9 weeks of recovery. One rat at 2500 ppm was found dead during week 18. Treatment-related cloudy eyes were observed at 100 and 2500 ppm during the treatment period. After 90 days, ophthalmoscopic examination revealed treatment-related hazy corneal opacity and/or corneal opacity with or without corneal vascularization at all dose levels; ophthalmoscopic examination of the recovery group animals indicated that all signs of corneal opacity and corneal vascularization had resolved by recovery week 9. A treatment-related decrease in adjusted mean body weight was observed at 2500 ppm, persisting throughout the 9-week recovery period. A statistically significant and dose-related increase in mean plasma tyrosine levels was observed at all dose levels.
throughout the treatment period with almost complete recovery at all dose levels observed at week 9 of the recovery period. A dose-related increase in liver and kidney weights adjusted to final body weight at all dose levels was observed in animals sacrificed at the end of the treatment period (90 days); these effects resolved in recovery group animals dosed with 5 and 100 ppm after 9 weeks but not in animals dosed with 2500 ppm. Microscopic examination and electron microscopy of liver and kidney tissues revealed no treatment-related abnormalities at 2500 ppm. Treatment-related induction of liver tyrosine aminotransferase (TAT) activity was observed after 90 days of treatment at 2500 ppm with complete recovery observed in the 9-week recovery group animals. Treatment-related inhibition of liver 4-hydroxyphenylpyruvate dioxygenase (HPPD) activity was observed after 90 days of treatment at all dose levels and persisting throughout the recovery period at all dose levels. Liver and kidney homogenate analyses revealed a dose-related increase in tyrosine levels in both kidney and liver at 5 and 100 ppm after the 90-day treatment period with complete recovery observed in the 2-week recovery group animals (these analyses were not conducted on the 2500 ppm animals). Possible adverse effect indicated- Ocular toxicity: corneal opacity was observed during clinical observations and at the 90-day ophthalmoscopic examination. NOEL (M) < 0.37 mg/kg/day (5 ppm) based on increased mean plasma tyrosine levels during treatment. Supplemental study (not a guideline study: only males were used, no hematology and no clinical chemistry analyses were conducted on the blood of the test animals, and microscopic examinations were conducted on liver and kidney tissues only). (Corlett and Leung, 10/21/2011)

Rabbit Repeated Dose 21-Day Dermal Toxicity Study
0018; 257932; “ZA1296: 21 Day Dermal Toxicity Study in Rabbits” (Lees, D., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Laboratory Project ID: Report No. CTL/P/5035, Study No. LB0575, 10/23/1997). 870.3200. ZA1296 (Batch reference number P17, purity = 96.8%) was moistened sufficiently with deionized water and applied to the clipped dorso-lumbar region of 5 New Zealand White albino rabbits per sex per dose at dose levels of 0 (deionized water only), 10, 500, and 1000 mg/kg/day for 6 hours for a total of 15 applications over a period of 21 days using an occlusive dressing. Treatment-related slight erythema was observed in females at 500 and 100 mg/kg/day and in males at 1000 mg/kg/day. No treatment-related mortalities occurred. No treatment-related effects on body weight or food consumption were observed. Ophthalmoscopy revealed no abnormalities. Hematology and blood clinical chemistry on the females revealed no treatment-related abnormalities. No treatment-related effects on organ weights were observed. Macroscopic and microscopic examinations revealed no treatment-related internal abnormalities. No adverse effects. Reported NOEL (M/F, systemic) = 1000 mg/kg/day based on no effects at the highest dose tested; NOEL (M, skin) = 500 mg/kg/day, NOEL (F, skin) = 10 mg/kg based on observed erythema. Unacceptable and not upgradable because no hematology and no blood clinical chemistry examinations were conducted on the male test animals. (Corlett and Leung, 10/27/2011)

The rabbit 21-day repeated dose dermal toxicity study was initially deemed to be unacceptable and not upgradeable because no hematology or clinical chemistry evaluations were performed on the male animals. Upon the request of the registrant, the study contents were re-evaluated in the context of whether the active ingredient presented a significant hazard with exposure via the dermal route. The study is still considered to be unacceptable due to the lack of the hematology and clinical chemistry data for males. However, several considerations preclude the request for having the study redone. In the three other species, rat, mouse and dog, for which hematology and clinical chemistry evaluations were performed (rec. nos. 257903, 257905, 257907, 257913), the hematology and clinical chemistry results in these subchronic studies did not demonstrate any remarkable difference in the response of either sex to the treatment with the active ingredient. In addition, the dermal penetration of the active ingredient was found to be quite minimal (rec nos. 258037, 258038, and 257039). At most, 1.8% of the applied dose penetrated the dermis up to 120 hours post-exposure in the rat in vivo study. It is unlikely that additional useful information would be gained by repeating the study in order to acquire the hematology and clinical chemistry data for
the male rabbits. These data should be sufficient to satisfy the requirement for a repeated dosing dermal toxicity study.

**Dog Subchronic Oral Toxicity Study**

0014; 257913; "ZA1296: 90 Day Oral Study in Dogs" (Brammer, A., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Laboratory Project ID: Report No. CTL/P/4945, Study No. PD1020, 10/01/1997). 870.3150. ZA1296 (Batch reference number P17, purity = 96.8%) was administered by gelatine capsules to 4 beagle dogs per sex per dose at dose levels of 0 (empty capsules), 100, 600, or 1000 mg/kg/day for at least 90 consecutive days. No mortalities occurred. Treatment-related lime-green colored urine, and staining of the coat, forelimb, forepaw, hindlimb, and hindpaw were observed in both sexes at 600 and 1000 mg/kg/day; treatment-related reddened ears were observed in males at 600 and 1000 mg/kg/day and in females at 1000 mg/kg/day. A treatment-related decrease in mean body weight (adjusted for initial weight) when compared to the control group was observed in males at 600 and 1000 mg/kg/day. Ophthalmoscopy revealed no treatment-related findings. A treatment-related increase in the mean red blood cell level and treatment-related decreases in mean cell volume and mean cell hemoglobin levels were observed in both sexes at 600 and 1000 mg/kg/day (microcytic polycythemia). Plasma chemistry revealed no treatment-related effects. No urinalysis data were submitted. No treatment-related effects on organ weights were observed. Macroscopic examination revealed treatment-related discolored (yellow) hair in both sexes at 600 and 1000 mg/kg/day. Microscopic examination revealed treatment-related focal mesothelial proliferation of the atrium of the heart in males at 1000 mg/kg/day; no other treatment-related abnormalities were observed in either sex. No adverse effects. Reported NOEL (M/F) = 100 mg/kg/day based on the presence of lime-green colored urine and microcytic polycythemia. Unacceptable but possibly upgradable with the submission of urinalysis data. (Corlett and Leung, 10/07/2011)

**Mouse 9-Day Dose Range-Finding Oral Toxicity Study**

53157-0024, 257951;" ZA1296: Dose Range Finding Study in Mice "; Moxon, M., 4/10/01. Central Toxicology Laboratory, Cheshire, UK SK10 4TJ, Laboratory Study ID: CTL Study #RM0739, Syngenta # 1252-98, Supplement to Rec# 257944. Groups of five female mice (Alpk:APfCD-1) were dosed by oral gavage with 0, 600, 800 or 1000 mg ZA1296/kg/day for up to 9 consecutive days to determine the highest dose level for the dose range finding study in the pregnant mice. Two mice in the 1000 mg/kg/day group and one in the 800 mg/kg/day group died during the study. No clinical signs were observed in any of the animals during the study. Similar increases in plasma concentration of tyrosine with time were reported in mice at 600 and 800 mg/kg/day. Peak tyrosine levels occurred from 4 to 6 hours post dose. The highest dose level for dose range finding study in the pregnant mice should be less than 800 mg/kg/day due to premature death of some mice in the 1000 and 800 mg/kg/day groups. Study Supplemental. (Pan and Leung, 6/2/11)

**Mouse 90-Day Dietary Toxicity Study**

0009, 257903; "ZA1296: 90 Day Dietary Dose Response Study in Mice" (Brammer, A. and Provan, W.M., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Report No. CTL/R/1316, Study No. XM6168, 12/19/1997). ZA1296 (Batch reference number P17, purity = 96.8%) was admixed to the diet and fed to 10 C57BL/10JfAP/Alpk mice per sex per dose at dose levels of 0 (untreated diet), 1, 10, 50, 100, 350, 1000, 3500, or 7000 ppm (0, 0.16, 1.7, 8.5, 18.0, 58.5, 179.3, 599.9, and 1222.5 mg/kg/day, respectively for males and 0, 0.19, 1.94, 10.8, 20.5, 72.7, 214.9, 714.8, and 1436.4 mg/kg/day, respectively for females) continuously for 90 consecutive days. An additional 10 animals per sex per dose level were included as satellite groups for interim sacrifice after 1 and 4 weeks of treatment. No treatment-related clinical signs were observed. A treatment-related slight decrease in adjusted mean body weight was observed at 7000 ppm in females. A statistically significant and dose-related increase in mean plasma tyrosine levels was observed in both sexes at all dose levels 10 ppm and above in weeks 1, 4, and 14. NMR analysis of urinary phenolic acids revealed a dose-related increase in urinary phenolic acid production up to
1000 ppm in males and up to 100 ppm in females with slightly lower concentrations at higher levels (p-hydroxyphenylpyruvic acid (HPPA) was the predominant phenolic acid present). No treatment-related effects on liver and kidney weights were observed. A treatment-related induction of liver tyrosine aminotransferase (TAT) activity was observed in males at week 1 at the 100 and 1000 ppm dose levels and at week 4 at the 350 ppm dose level but was not observed at week 13; a treatment-related induction of liver TAT activity was observed in females at all dose levels at weeks 1, 4, and 13. A treatment-related inhibition of liver p-hydroxyphenylpyruvate dioxygenase activity was observed in both sexes at all dose levels at weeks 1, 4, and 13. **No adverse effects indicated.** NOEL not determined. **Supplemental study** (not a guideline study: no hematology and no clinical chemistry were conducted on serum of the test animals and no macroscopic and no microscopic examinations of the internal organs of the test animals were performed). (Corlett and Leung, 08/17/2011)

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**Mouse 28-Day Dietary Immunotoxicity Study**

**53157-0107; 25796**; “Mesotrione - A 28-Day Dietary Immunotoxicity Study in CD-1 Female Mice”; (J.M. Wasil, V.L. Peachee; WIL Research Laboratories, LLC, Ashland, OH and ImmunoTox, Inc., Richmond, VA; Study No. WIL-639174; 2/17/12); Ten CD-1 female mice/group received 0, 500, 1500 or 5000 ppm of Mesotrione technical (batch no. 631795; purity: 83.0%) in the diet for 28 days (0, 110.3, 332.4, 1167.7 mg/kg/day). Another 10 females were dosed by intraperitoneal injection with 50 mg/kg of cyclophosphamide on study days 24 through 27 as the positive control group. On day 24, each animal received an iv injection of 1x10^8 sheep red blood cells (SRBC) (0.2 ml volume). SRBC specific IgM plaques were determined for each animal by incubating a spleen cell suspension preparation with guinea pig complement and SRBC. No deaths occurred during the treatment period. There was no apparent treatment-related effect upon the mean body weights or food consumption. There were no treatment-related lesions noted in the necropsy examination. There was no treatment-related effect upon the thymus or spleen weights. **No adverse effect indicated.** The positive control was functional. **Study acceptable.** (Moore, 7/3/12)

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**MODE OF ACTION**

53157-0029, 257968;" ZA1296: Biochemical studies in rat and mouse liver"; Odum, J., 11/14/97. Central Toxicology Laboratory, Cheshire, UK, Laboratory Project ID: CTL/R/1339. Groups of CD rats (Sprague-Dawley) and CD1 mice (Swiss) were exposed to ZA 1296 in the diets at 1000, 7000, and 16000 ppm (rats) or 1000, 3000, and 7000 ppm (mice) for 28 days. Each treatment group contained 5 animals and control groups contained 10 animals each. Standard inducers for CYP 450, β-naphthoflavone, Phenobarbital, dexamethasone and methyl clofenapate were administered to Alpk:AP rats (3/group) and AP Swiss albino mice (4/group) by i.p. daily for 4 days. At the end of
the exposure, animals were killed and livers were removed, weighed and the liver microsomal fractions were prepared individually (for animals given ZA1296 or controls) or pooled according to group (for animals given standard inducers). CYP 450 activities and CYP isoenzyme profiles were determined. There were no ZA1296 treatment related changes in body weight, food consumption, clinical observation and clinical chemistry parameters during or at the end of the study. Liver pathological examinations revealed no adverse histopathology in the ZA1296 treated rats and mice. The changes of absolute and relative liver weights were insignificant or minimal compared with significant or marked increases in rats and mice treated with standard inducers. The inductions of CYP 450 activities and effects on CYP isoform profiles by ZA1296 treatments were minimal compared with the inductions by standard inducers. Study Supplemental. (Pan, 8/4/11)

53157-0031; 257973, 257975; “(Mice) Investigation of Liver and Kidney Enzyme Parameters in Control Mouse Pups from New Born to Age 42 days, (Rats) Investigation of Liver and Kidney Enzyme Parameters in Control Rat Pups from New Born to Age 42 days; (Mice: J. Williams, (Rats: M.E. Moxon; (Mice) Study No. RM0801, (Rats) Study No. RR0798; (Mice) 1/23/01, (Rats) 11/16/00); The tyrosine aminotransferase (TAT) and hydroxyphenylpyruvate dioxygenase (HPPD) activities were measured in the liver and kidneys of untreated CD1 mice and Sprague-Dawley rats ranging in age from 1 day to 42 days. Blood was collected from each of the study animals and plasma tyrosine levels were measured as well. In addition, the organs and tissues of the dams for each of the litters which were euthanized, were dissected and analyzed for these respective parameters. The plasma tyrosine levels in the young pups of both sexes of both species were approximately the same magnitude (80 to 270 nmol/ml) and varied in a corresponding manner with plasma levels increasing between postnatal day 5 and 12 and then declining thereafter. The tyrosine level of the dams in both species ranged between 50 and 100 nmol/ml throughout the study period. TAT and HPPD activities were greater in the liver cytosol of both species and there were no apparent sex-related differences in the magnitude of activity of either enzyme for the pups. The liver TAT activity level was greater in the mice dams than the level observed for the rats (6.18 to14.01 vs. 0.21 to 4.21 nmol HPPA/min/mg protein, respectively). In addition, HPPD activity in the liver of the mice ranged up to 10 to 20 fold greater than the activity observed in the kidneys. These data should be useful for ascertaining the importance of the respective enzymes in affecting the relative toxicity of mesotrione in these species. Study supplemental. (Moore, 6/10/11)

53157-0032; 257977; “A Study to Investigate the Morphology and Pathology of Ocular Lesions in Alpk:ApfSD Rats Fed Different Levels of Tyrosine in a Low Protein Diet”; (M. Robinson; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Report No. CTL/R/1243; 6/21/95); Eight weanling (3 to 4 weeks old) male Sprague Dawley rats/group received 0, 0.5, 1.0, 2.5 or 5.0% tyrosine in the diet for 3 weeks. Their eyes were examined prior to the initiation of the study and on study days 2, 3, 4, 5, 6, 7, 8, 11, 12, 14, 18 and 21 of the study by means of indirect ophthalmoscopy. Corneal lesions were apparent in the eyes of rats in both the 2.5 and 5.0% treatment groups within the 1st week of treatment. The earliest onset was day 3 in 5 animals in the 2.5% group. The lesions initially developed as small focal areas of opacity on the corneal surface which then coalesced into larger areas of opacity. None of the other study animals developed ocular lesions during the 21-day treatment period. Study supplemental. (Moore, 6/10/11)

53157-0036; 257992; “Mesotrione-Exposure Study in Alpk:APfCD-1 Mouse”; (A. Duerden; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. RM1026; 10/8/07); Twenty five-mated CR-1 female mice/group received 0, 10, 100, 1000 or 7000 ppm of Mesotrione (technical wet paste) (batch no. CCR4C13A; purity: 82.9%) in the diet from day 6 of gestation up to day 23 post-partum (gestation: 0, 1.6, 15.8, 154.5, 1076.9 mg/kg/day, post-partum: 0, 3.5, 37.0, 359.8, 2238.4 mg/kg/day). Five dams/group/time point were euthanized on gestation day 19 and post partum days 5, 12, 18 and 23. Blood was collected from each of the dams, the fetuses on gestation day 19 and the pups at the ensuing time points and the plasma mesotrione and
tyrosine levels were measured. No unscheduled deaths occurred during the study. The 7000 ppm dams demonstrated lower mean body weights at various time points during the post-partum period in comparison with the control group. The mean food consumption was consistently less than that of the control group during the post-partum period. The mean body weights of the pups in the 7000 ppm group were less than those of the control group over the course of the post-partum period. The plasma tyrosine levels corresponded qualitatively with increases in a.i. uptake in the dams (i.e., increased a.i. uptake during the post-partum treatment period resulted increased plasma levels of tyrosine). The overall plasma tyrosine levels in the dams and pups appeared to approach a plateau at the higher treatment levels. The plasma mesotrione levels confirmed that the uptake of the a.i. corresponded reasonably well with the treatment levels in the feed. Possible Adverse Effect: excessive levels of tyrosine in the plasma. Maternal NOEL: <10 ppm (<1.6 mg/kg/day) (based upon elevated tyrosine levels in the plasma of the 10 ppm dams during the gestation period); Pup Developmental NOEL: < 10 ppm (< 1.6 mg/kg/day) (based upon elevated tyrosine levels in fetuses during gestation) Study supplemental. (Moore, 6/29/11)

53157-0041; 258001; “Nitisinone - Pre and Postnatal Development Study in the Mouse”; (M.E. Moxon; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. RM0960; 5/5/04); Twenty four mated female Alpk:APfCD-1 mice/group were dosed orally by gavage with 0 (vehicle: aqueous 1% (w/w) carboxymethylcellulose with phosphate buffer (pH 6.8), 5, 50 or 250 mg/kg/day of Nitisinone (triketone analogue of mesotrione) (batch no. 0556865; purity: 100%) from day 7 of gestation through day 21 post-partum. Upon completion of the treatment period, 20 F1 offspring/sex/group were maintained on study through a 10-week premating period, up to 2 weeks for mating and for 14 days of gestation. The neurodevelopment of the F1 generation was assessed by means of a functional observational battery, surface righting reflex, olfactory discrimination, and a tail flick tests, learning and memory testing in a maze and a motor activity assessment. The time to manifestation of developmental landmarks was also recorded. The F0 dams survived the treatment. Among the F1 offspring selected as parents, one male and one female in the 250 mg/kg group were euthanized in extremis by day 22 post-partum due to poor health. There was no treatment-related effect on the body weights of the dams in the F0 generation. However, the mean food consumption of the 250 mg/kg dams was less than that of the control group over the course of the treatment period (p<0.01 or 0.05). The length of gestation was significantly increased for 50 and 250 mg/kg groups (p<0.05). The mean body weights of both the male and female offspring of the 250 mg/kg in the F1 generation were less than the control group during the lactation period (p<0.01 or 0.05). This effect persisted in the females through the 10-week premating period. The relative survival of the F1 pups during the lactation period was less than that of the control group (p<0.05). The percentage of both sexes in the 250 mg/kg group which could successfully complete the olfactory discrimination test on day 16 post-partum was less than that of the control group (p<0.05). In both the learning and memory tests, both sexes in the 250 mg/kg of the F1 generation demonstrated a diminished ability to complete the task as quickly as the offspring in the control group did (p<0.01 or 0.05). No treatment-related effects were noted in the functional observational battery, the time to manifestation of developmental landmarks, the surface righting reflex and the tail flick tests and the motor activity assessment. Possible adverse effect: diminished learning and memory; Mouse Developmental Neurotoxicity NOEL: (M/F) 50 mg/kg/day (based upon the diminished learning and memory of the 250 mg/kg F1 generation). Study supplemental (study did not fulfill guidelines of a Developmental Neurotoxicity Study (836)). (Moore, 8/18/11)

53157-0035; 257990; “ZA1296: Ocular Toxicity Development and Reversibility Study in Rats”; (D.J. Tinston; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. PR1022; 4/23/97); Sixteen and 40 male Sprague-Dawley rats received 0 or 2500 ppm, respectively, of ZA1296 (Mesotrione Technical) (batch no. P17; purity: 96.8%) in the diet for 5
weeks (0, 272 mg/kg/day). At the conclusion of this treatment period, a recovery cohort of 8 control and 15 treated animals were maintained on control diet for another 8 weeks. During the study and recovery period, the eyes of each study animal were examined by indirect ophthalmoscopy. The mean body weight of the treated animals was less than that of the control animals during the treatment period. Food consumption was also less during this period for the treated group. The incidence of ocular lesions in the treated animals increased over the course of the treatment period with 39 of the 80 observed eyes in this group demonstrating corneal opacity by the end of the treatment period. The severity ranged from slight to marked. Seven of the cornea were noted to be vascularized. By week 7 of the recovery period, one of the 30 eyes which were examined demonstrated a slight hazy opacity and ghost vascularization was noted in eleven others. The mean plasma tyrosine level peaked at 2956 nmol/ml during the treatment period and gradually returned to the control level by the conclusion of the recovery period. In the histopathological evaluation of the eye, at the termination of the treatment, keratitis was noted in the eyes of 11 out of 25 treated animals which ranged from minimal to moderate. Polymorphonuclear leucocytic infiltration was evident in the eyes of ten treated animals which ranged in severity from minimal to moderate. At the conclusion of the recovery period, corneal vessels were evident in the eyes of 8 out of 15 treated animals which ranged from minimal to slight in severity. Corneal stromal fibroblasts were noted in the eyes of 7 of these animals as well as in an additional animal in this group. The severity ranged from minimal to slight. Corneal epithelial disruption was also noted for three animals. Of the 15 treated animals examined in this cohort, the eyes of 6 of them were free of lesions. Possible adverse effect: residual lesions in the cornea of the eye. Supplemental study (non-guideline). (Moore, 6/22/11)

STUDIES ON METABOLITES

NMBA

“NMBA: 28-Day Oral Toxicity Study in Rats” (Milburn, G.M., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, Report No. CTL/P/5578, Study No. KR1281, 01/12/1998). NMBA (2-nitro-4-methylsulphonylbenzoic acid, Batch reference WRC 15483-30-1, purity = 97.1%) was suspended in corn oil and administered by gavage to 5 Alpk:APfSD rats per sex per dose at dose levels of 0 (corn oil only), 15, 150, or 1000 mg/kg/day for 28 consecutive days. No treatment-related mortalities occurred during the study. No treatment-related clinical signs were observed. No effect on body weight or food consumption was observed. Functional observational battery (FOB) was conducted during week 4 of the study. No treatment-related effects on time to tail flick, landing foot splay or grip strength were observed. Motor activity assessments conducted during week 4 revealed a treatment-related increase in mean activity in females at 150 and 1000 mg/kg/day. Hematological and serum chemistry investigations revealed no treatment-related effects. Differences in organ weights were not considered toxicologically significant in the absence of histological changes. Macroscopic and microscopic examinations of the test animals revealed no treatment-related abnormalities. No adverse effects. NOEL (M) = 1000 mg/kg/day (based on no effects at the highest dose tested), NOEL (F) = 15 mg/kg/day (based on increased activity during motor activity assessments). Supplemental study (test article was a metabolite of the active ingredient mesotrione). (Corlett and Leung, 06/29/2011)

53157-0042 258004, “MNBA: An evaluation of mutagenic potential using S. Typhimurium and E.coli” 842; Central Toxicology Laboratory, Cheshire, UK, Laboratory Project ID: CTL/P/4955, 4/22/96; Callander, R. D. MNBA: CTL ref#: Y08636/003, Batch ref: WRC15483-30-1, a white solid with purity of 97.0% w/w. MNBA was tested in 4 strains of S. Typhimurium (TA 1535, TA1537, TA 98, and TA 100) and two strains of E.coli (WP2P and WP2P uvrA) for its ability to cause reverse mutation with or without metabolic activation. Six concentrations of the test substance were tested: 5000, 2500, 1000, 500, 200 and 100 ug/plate. In the phase I of the study, plate-incorporation method was used for the 6 strains under metabolic activation and no activation, in phase II,
plate-incorporation method was used for the tests done without metabolic activation and pre-incubation method was used for tests done with metabolic activation. The test substance did not induce statistically significant increase of revertant colony numbers at the 6 concentrations tested under metabolic activation or no activation except for a few incidental changes. Positive control materials induced expected increases in mean revertant colony number per plate over that of the negative controls. Study acceptable. (Pan and Leung, 8/12/11).

AMBA

53157-0032; 257979, 257981; “ZA1296: Effects of AMBA, a Metabolite of ZA1296 on p-Hydroxyphenylpyruvate Dioxygenase (HPPD) Activity; ZA1296: Effects of MNBA, a Metabolite of ZA1296 on p-Hydroxyphenylpyruvate Dioxygenase (HPPD) Activity; (B.M. Elcombe, S. Meadowcroft; Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study Nos. XR6243, XR6244; 4/16/98); The potential of ZA1296 (batch no. P17, purity 96.8%), two of its metabolites, AMBA (2-amino-4-methylsulphonyl benzoic acid) (purity: 99%) and MNBA (4-methylsulphphonyl-2-nitro-benzoic acid) (purity: 97%), and the therapeutic agent, NTBC (nitro-4-(trifluoromethyl)benzoyl]-4,4,6,6-tetramethyl-cyclohexane -1,3,5-trione) (purity: 92%) to inhibit HPPD activity in vitro in liver homogenates derived from male Sprague-Dawley rats was evaluated. Both ZA1296 and NTBC inhibited the enzyme at equimolar concentrations, with 100% inhibition at 20 uM. AMBA and MNBA demonstrated less potential to inhibit HPPD under the assay conditions employed. Study supplemental. (Moore, 6/10/11)

53157-0042 258006, “AMBA (2-amino-4-methylsulfonyl Benzoic acid): An evaluation of mutagenic potential using S. Typhimurium and E.coli” 842; Central Toxicology Laboratory, Cheshire, UK, Laboratory Project ID: Report #: CTL/P/5226, Study No: YV3941, 11/28/96; Callander, R. D. AMBA: CTL ref#: Y09476/001, Batch ref: WRC16010-09-01, a yellow solid with purity of 99% w/w. AMBA was tested in 4 strains of S. Typhimurium (TA 1535, TA1537, TA 98, and TA 100) and two strains of E.coli (WP2P and WP2P uvrA) for its ability to cause reverse mutation with or without metabolic activation. Six concentrations of the test substance were tested: 5000, 2500, 1000, 500, 200 and 100 ug/plate. In the phase I of the study, plate-incorporation method was used for the 6 strains under metabolic activation and no activation, in phase II, plate-incorporation method was used for the tests done without metabolic activation and pre-incubation method was used for tests done with metabolic activation. The test substance did not induce significant increase of revertant colony numbers at the 6 concentrations tested under metabolic activation or no activation except for a few incidental changes. Positive control materials induced expected increases in mean revertant colony number per plate over that of the negative controls. Study acceptable. (Pan and Leung, 8/16/11).