APPENDIX J: HED Effects Tables

The following information is from:

USEPA (2007). Alachlor: PP#8F05000 and 8F5025. FQPA Human Health Risk Assessment for Section 3 New Uses on Cotton, Sunflower, and for Inadvertent Tolerances on Various Rotational Crops (Cereal Grains and Nongrass Animal Feeds). PC Code: 090501. DP Barcode D330812, 247976. Office of Pesticide Programs, Washington, DC. January 8, 2007.

Table 1. Acute Toxicity Profile (Alachlor).

Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral [rat]	00139383	LD_{50} = 930 mg/kg	III
870.1200	Acute dermal [rabbit]	00139384	LD_{50} = 13.3 g/kg	IV
870.1300	Acute inhalation [rat]	00109561	LC_{50} = 1.04 mg/L (4 hours)	III
870.2400	Acute eye irritation [rabbit]	00139385	No significant irritation	IV
870.2500	Acute dermal irritation [rabbit]	00139386	No significant irritation	IV
870.2600	Skin sensitization [guinea pig]	00161728	Sensitizer	n/a

Table 2. Subchronic, Chronic, and Other Toxicity Profile.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100	90-Day oral toxicity (rat)	No acceptable study submitted, satisfied by 870.4100a.	
870.3150	6-Month oral toxicity (beagle dog)	00087479 (1981) 0, 5, 10 or 25 mg/kg/day (capsule) Acceptable/guideline	NOAEL = not established (<5.0 mg/kg/day) LOAEL = 5 mg/kg/day based on increased liver weight (liver toxicity at higher doses included microscopic lesions).
870.3200	21-Day dermal toxicity (New Zealand White rabbit)	00147328 (1985) 0, 50, 300 or 1000 mg/kg/day on skin Acceptable/guideline	NOAEL = 50 mg/kg/day LOAEL = 300 mg/kg/day based on hematological/clinical chemistry alterations.
870.3700a	Prenatal developmental in (rat)	00043645 (1980) 0, 50, 150 or 400 mg/kg/day by gavage Acceptable/guideline	**Maternal** NOAEL = 150 mg/kg/day LOAEL = 400 mg/kg/day based on increased clinical signs, mortality and decreased body weight. **Developmental** NOAEL = 150 mg/kg/day LOAEL = 400 mg/kg/day based on increased postimplantation loss, reduced numbers of live fetuses.
870.3700b	Prenatal developmental in (New Zealand White rabbit)	40579401, -02 (1988) 0, 50, 100, 150 mg/kg/day by gavage Acceptable/guideline	**Maternal** NOAEL = 100 mg/kg/day LOAEL = 150 mg/kg/day based on decreased body weight gain during treatment period. **Developmental** NOAEL = 150 mg/kg/day LOAEL = not established (>150 mg/kg/day).
870.3800	Reproduction and fertility effects (rat)	00075062 (1981) 0, 3, 10 or 30 mg/kg/day in diet Acceptable/guideline	**Parental/Systemic** NOAEL = 10 mg/kg/day LOAEL = 30 mg/kg/day based on renal toxicity. **Reproductive** NOAEL = 30 mg/kg/day LOAEL = not determined (>30 mg/kg/day). **Offspring** NOAEL = 10 mg/kg/day LOAEL = 30 mg/kg/day based on renal toxicity.
870.4100a	Chronic toxicity (Long-Evans rat)	00141060 (1984); 40284001 (1987) 0 or 126 mg/kg/day administered in the diet for two years. Group of animals treated for 5-6 months, then sacrificed at two years. Acceptable/nonguideline (special study intended to evaluate uveal pathology)	NOAEL = not established (>126 mg/kg/day) LOAEL = 126 mg/kg/day based on ocular lesions (uveal degeneration syndrome).

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.4100b	Chronic toxicity (beagle dog)	00148923 (1984) 0, 1.0, 3.0 or 10.0 mg/kg/day in diet Acceptable/guideline	NOAEL = 1.0 mg/kg/day LOAEL = 3.0 mg/kg/day based on hemosiderosis in kidney/spleen (males).
870.4200	Carcinogenicity (CD-1mouse)	00075709 (1981) 0, 26, 78 or 260 mg/kg/day, administered in the diet for 18 months. Acceptable/guideline	NOAEL = 26 mg/kg/day in males; 78 mg/kg/day in females. LOAEL = 78 mg/kg/day in males based on thyroid follicular atrophy and increased liver and kidney weights;260 mg/kg/day in females based on increased mortality, decreased body weight gains and thyroid follicular atrophy. **Bronchioalveolar adenomas and/or carcinomas in lungs of male and female mice not included in cancer classification due to inconsistencies in dose-response.**
870.4200	Carcinogenicity (CD-1 mouse)	43507601 (1994) 0, 16.6, 65.4 or 262.4 mg/kg/day, males; 0, 23.7, 90.3 or 399.2 mg/kg/day, females in diet for 18 months. Acceptable/guideline	NOAEL = 16.6 mg/kg/day in males; 90.3 mg/kg/day in females LOAEL = 65.4 mg/kg/day in males based on increased centrilobular hepatocellular hypertrophy; 399.2 mg/kg/day in females based on decreased body weight gain and increased fibrous osteodystrophy of the sternum. **Bronchioalveolar adenomas and/or carcinomas in lungs of male and female mice not included in cancer classification due to inconsistencies in dose-response.**
870.4300	Chronic toxicity/carcinogenicity (Long-Evans rat)	00091050 (1981) 0, 14, 42 or 126 mg/kg/day, administered in the diet for 117 weeks to males and 107 weeks to females. Acceptable/guideline	NOAEL = not determined (<14 mg/kg/day) LOAEL = 14 mg/kg/day based on uveal degeneration syndrome and increased thyroid weights. **Increased incidence of nasal olfactory epithelial adenomas/adenocarcinomas in males and females; thyroid follicular cell and gastric tumors also increased.**
870.4300	Chronic toxicity/carcinogenicity (Long-Evans rat)	00139021 (1984) 0, 0.5, 2.5 or 15 mg/kg/day, administered in the diet for 110 weeks. Acceptable/guideline	NOAEL = 2.5 mg/kg/day LOAEL = 15 mg/kg/day based on uveal degeneration syndrome and increased mortality in females; abnormal disseminated foci of the liver in males. **Increased incidence of nasal olfactory epithelial adenomas/carcinomas observed in males and females. Significant at 15 mg/kg/day; single tumor**

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
Bacterial Gene Mutation 870.5100	Ames bacterial reverse mutation	00109563 (1980) 10 - 5000µg/plate Acceptable/guideline	Negative for *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 or TA100 in presence or absence of S9.
Bacterial Gene Mutation 870.5100	Ames *E. coli* bacterial reverse mutation	00109563 (1980) 10 - 5000 µg/plate Acceptable/guideline	Negative for *E. coli* WP2 hcr reverse mutation assay in presence or absence of rat liver S9.
Bacterial Gene Mutation 870.5100	Ames bacterial reverse mutation	42651301 (1990) 50 - 5000 µg/plate alachlor; also tested DMTA and DEA metabolite intermediates Acceptable/guideline	Alachlor negative for *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 or TA100 in presence or absence of S9 from uninduced rat, mouse or monkey nasal turbinates. Diethyl-2-methylthioacetanilide (DMTA), proposed metabolic intermediate of alachlor during metabolism to s-methylsulfoxide was positive (marginal positive response) in strain TA1535 with mouse nasal turbinate S9; marginal increases with rat nasal turbinate S9. 2', 6'-diethylaniline, proposed toxic metabolite of alachlor, was positive (marginal positive response) in TA 1535 and TA100 with mouse nasal turbinate S9, in strain TA100 with rat nasal turbinate S9.
Bacterial DNA Damage/Repair 870.5500	*B. subtilis* rec assay	00109563 (1980) 20 - 2000 µg/plate Acceptable/guideline	Negative in *B. subtilis* strains M45 and H17.
Mammalian Gene Mutation 870.5300	Chinese hamster ovary (CHO) cell HGPRT forward mutation *in vitro*	00148921 (1984) 15 - 150 µg/plate (-S9); 15 - 330 µg/plate (+S9) Acceptable/guideline	Negative for gene mutation in presence or absence of S9.
Mammalian *in vivo* Cytogenetics 870.5385	Structural chromosomal aberrations in rat bone marrow	00141062 (1984) Single gavage doses of 0, 100, 300 or 1000 and sacrifice at 6, 12, 24 and 48 hrs. Acceptable/guideline	Negative for chromosomal aberrations in rat bone marrow.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
Mammalian *in vivo* erythrocyte micronucleus 870.5395	Rat bone marrow micronucleus assay	42651303 (1992) Single injections of 0, 150, 300 or 600 mg/kg and sacrifice at 24, 48 and 72 hrs. Acceptable/guideline	Negative in Long-Evans rats for clastogenic or aneugenic effect. Test material reached target organ (demonstrated radioactively).
Mammalian *in vivo* erythrocyte micronucleus 870.5395	Mouse bone marrow micronucleus assay	44032103 (1995) Single gavage doses of 250, 500 or 1000 mg/kg and sacrifice at 24 and 48 hrs. Acceptable/guideline	Negative in CD-1 mice up to lethal doses.
Unscheduled DNA synthesis 870.5550	Unscheduled DNA synthesis assay in cultured mammalian cells (rat hepatocytes)	00141061 (1984) 50, 200 and 1000 mg/kg by gavage; hepatocytes harvested at 2 and 12 hrs. Acceptable/guideline	Positive for UDS in rat liver cells at 1000 mg/kg
Unscheduled DNA synthesis 870.5550	Unscheduled DNA synthesis assay in cultured mammalian cells (rat hepatocytes)	42651302 (1992) 1000 mg/kg by gavage; hepatocytes harvested at 12 hrs. Acceptable/guideline	Positive for UDS in rat liver cells at 1000 mg/kg.
Other Effects (nonguideline)	Comet assay in nasal cells of the rat	44203701 (1996) 126 mg/kg/day in the diet for 1 week Acceptable/guideline	Negative for DNA damage in isolated rat nasal epithelial cells.
870.7485	Metabolism and pharmacokinetics (Sprague-Dawley rat)	00132045 (1983) 7 and 700 mg/kg, single gavage doses Acceptable/guideline	Biphasic elimination via urine and feces. Initial phase half life of 0.1 to 10.6 hrs followed by a slow phase of 5 to 16 days (89% of administered dose was eliminated by 10 days). Total of 14 metabolites identified in urine and 13 in feces; 3 common to both. Metabolites were conjugates of mercapturic acid, glucuronic acid and sulfate. Some tissue bioaccumulation observed.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
870.7485	Metabolism and pharmacokinetics (female Long-Evans rat)	42651306, -08; 42852107, -08 (1996) Groups 1-3, 33 rats/group given 7, 70 or 700 mg/kg radiolabeled alachlor, single doses; Group 4 - 21 rats given 15 doses of 700 mg/kg; Group 5 – 6 rats given single oral dose of 700 mg/kg for plasma samples at 2, 4 and 6 hrs postdosing. Acceptable/guideline	Oral studies: absorption almost complete, not affected by repeated dosing. Residual radioactivity 5% or less of dose in all groups. Nasal turbinates showed secondary peak of radioactivity at 8 hrs postdosing at both levels. Excretion was by both urinary (30-47%) and fecal (41-45%) route, with repeated oral dosing at high dose resulting in slightly increased fecal excretion. Major urinary metabolite was sec-amide hydroxymethyl sulfone and major fecal metabolites were the tert-amide mercapturic acid and disulfide, which also showed increases at the higher dose.
870.7485	Metabolism and pharmacokinetics (Rhesus monkey)	40000901 (1986) 0.7, 7.0 mg/kg IV Acceptable/nonguideline	Excretion largely complete by 3 days postdosing. Ratio of fecal:urinary metabolites 9-10:1. Of six urinary metabolites identified, two are also seen in the rat (secondary and tertiary mercapturic acid conjugates). One fecal metabolite was identified, also in urine. One of the urinary metabolites was mutagenic in Ames assay.
870.7600	Dermal penetration (monkey)	00149403, 00149404, 00149405 (1984, 1985) Acceptable/nonguideline	Dermal penetration *in vivo* estimated to be 24% of administered dose.
Special studies (nonguideline)			
n/a	Thyroid mechanistic study (Long-Evans rats)	42957201 (1993) 0 or 126 mg/kg/day in diet for 7, 14, 28, 60 or 120 days and one 60-day group followed by a 60-day recovery period. Acceptable/nonguideline	Increased liver and thyroid weights throughout study; increased liver UDPGT activity and TSH levels from Day 14; T3 increased and T4 decreased, then increased. Thyroid follicular cell hypertrophy/hyperplasia seen in 28 and 60 day groups, one in 120-day group. Recovery group similar thyroid hormone levels to controls.
n/a	Metabolism-effects of multiple oral dosing (Long - Evans rats)	42651310 (1989), 42852109, (1988) 0.5, 2.5, 15, 42 or 126 mg/kg radiolabeled alachlor for 9 days Acceptable/nonguideline	Metabolic pathways for alachlor not significantly altered by repeated dosing but enzyme activities may be altered at higher dose levels, with increased sulfide conjugates observed in feces at higher doses.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
n/a	Metabolism of alachlor in rats chronically exposed to alachlor (Long-Evans rats)	42651307 (1986), 42931101 (1986) 0, 0.5, 14 or 126 mg/kg/day in the diet for 16 months or for 4 weeks; single radiolabeled dose at end of study. Acceptable/nonguideline	Males exposed long-term showed increased fecal/decreased urinary excretion of radioactivity than males exposed short-term; fecal radioactivity lower in short-term females than males. Nine major urinary, 1 major fecal metabolite identified. Some differences identified in metabolism among preconditioned and nonpreconditioned animals, leading to increased formation of hydroxylated metabolites. Increased disulfide metabolite seen in feces at 126 mg/kg.
n/a	Metabolism of alachlor in the mouse (CD-1 mice)	42651305, -06 (1985, 1986) 890 mg/kg males, 819 mg/kg females Acceptable/nonguideline	Feces major route of excretion. Feces showed higher levels of cysteine and glucuronide conjugates than rat. Ten fecal metabolites (major were tert-amide mercapturic acid; tert-amide cysteine conjugate and NCH2O-glucuronide) and 7 urinary metabolites (major was NCH2O glucuronic acid) identified.
n/a	Metabolism of alachlor methyl sulfide (Long-Evans rat)	42651309 (1988) 0.73 or 7.93 mg/kg radiolabeled alachlor methyl sulfide, sacrifice at 24 and 120 hours postdose Acceptable/nonguideline	Most radioactivity excreted via urine at both dose levels. Major metabolite was sulfone metabolite of alachlor. The metabolite 4-amino-3,5-diethylphenylsulfate was also seen, which is a potential precursor product for formation of cytotoxic quinine imine metabolite.
n/a	*In vitro* metabolism comparative liver slice assay (rat, mouse, two male and one female monkey)	42651311 (1986) Incubations of liver slices at 0.05 or 0.5 radiolabeled alachlor and analysis of % alachlor metabolized and rate of metabolism at 2 and 4 hours. Acceptable/nonguideline	Although overall rate of metabolism of alachlor was similar among species, rate of metabolism in female monkey was about twice that of males or of other species. Metabolism appeared to be rate-limiting after 2 hours at the 0.5 mM concentration. Polar metabolites and an unknown metabolite (#2) major in all species at 2 hours but hydroxyl-alachlor and secondary amide metabolites were major at 0.5 mM. GSH conjugate lower in all species at high vs. low concentration, with monkey producing less than rat or mouse. Hydroxy-alachlor metabolite increased with dose in all species. Mouse liver at low concentrations and 4 hours produced higher levels of GSH conjugate and unknown metabolite #1 than rat, but rat made more unknown #2.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
n/a	*In vitro* metabolism from tissue fractions of rat nasal turbinates, liver, kidney, lung, glandular stomach, and nonglandular stomach	42651312 (1988); 42852110 (1977) Preparation of tissue fractions; incubation of S9 fractions at 0 2mM alachlor, 0.2 mM methylsulfide conjugate of alachlor, or 0.05 mM 2,6-diethylaniline metabolite. Acceptable/nonguideline	Analysis of homogenates and microsomal fractions of rat liver, kidney, lung, glandular stomach, non-glandular stomach and nasal turbinates showed that levels of enzymes in tissue preparations were in general agreement with published values. Fractions were analyzed to study *in vitro* metabolism of alachlor (0.2 mM) and further metabolism of alachlor metabolites methylsulfide conjugate of alachlor (0.2 mM) and 2,6-diethylaniline (0.05 mM). Overall, the rate of sulfate conjugation in nasal tissue is about an order of magnitude slower than in liver. Increased formation of the phenol metabolite together with reduced glutathione levels in nasal tissue versus liver may make this metabolite available longer in nasal versus liver tissue.
n/a	*In vitro* metabolism of alachlor (rat and mouse liver, nasal tissues)	42852111 (1988) Comparison of liver and nasal tissues from rats and mice to metabolize alachlor to proposed DEBQI reactive intermediate. Acceptable/nonguideline	Report summarizes several metabolism studies on alachlor in various species. Study proposes that conversion of alachlor metabolites to reactive intermediate 2,6-diethylbenzoquinoneimine (DEBQI) is greater in rat than mouse nasal tissue based on high levels of enzyme activities in rat nasal tissues. The secondary amide methylsulfide metabolite of alachlor is believed to be the actual substrate for formation of the DEBQI intermediate by hydrolysis by arylamidases to diethylaniline, which is sequentially oxidized to DEBQI.
n/a	*In vivo* metabolism of alachlor methyl sulfide (Long-Evans rats)	42651309 (1988) Female rats received 0.7 or 7.0 mg/kg radiolabeled alachlor methyl sulfide by gavage. Acceptable/nonguideline	As a percentage of dose, 0.86% and 1.71% of the 4-amino-3,5-diethylphenyl sulfate was formed at the 0.7 and 7.0 mg/kg dose, respectively.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
n/a	*In vitro* metabolism of alachlor and alachlor metabolites by rat and monkey nasal turbinates	42651314 (1990) *In vitro* metabolism of alachlor, alachlor secondary methyl sulfide and 2,6-diethylaniline by cytosolic or microsomal fractions from liver and nasal turbinates of rats and monkeys. Acceptable/nonguideline	Metabolism of alachlor by glutathione transferase was 3.9 times greater in rat than monkey liver and 114.3 times greater in rat than monkey nasal tissue; hydrolysis of secondary sulfide by arylamidase in rat and monkey liver was similar but was 4 times greater in rat than monkey nasal tissue; hydroxylation of 2,6-diethylaniline by arylhydroxylase was 3 times greater in rat than monkey liver and 7.6 times greater in rat than monkey nasal tissue. Enzymes responsible for conversion of alachlor to DEA and hydroxylation of DEA therefore more active in rat.
n/a	*In vitro* metabolism of alachlor and alachlor metabolites by rat and human nasal turbinates and liver	43482301 (1994) *In vitro* metabolism of alachlor, alachlor secondary methyl sulfide and 2m6-diethylaniline by cytosolic or microsomal fractions from liver and nasal turbinates of rats and humans (postmortem samples). Acceptable/nonguideline	Conjugation of alachlor with glutathione by glutathione transferase was 4.0 times greater in rat than human liver and 32.5 times greater in rat than human nasal turbinate tissue; hydrolysis of alachlor secondary sulfide was 5.8 times greater in rat than human nasal tissue; hydrolysis of alachlor secondary amide was 3.7 times greater in rat than human nasal tissue; in liver, there was measurable activity in the rat but not in humans; the hydroxylation of DEA was 7.5 times greater in rat than human liver and 129.8 times greater in rat than human nasal tissue. Conversion of alachlor to DEA-phenol metabolite therefore low for humans in comparison to the rat.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
n/a	*In vivo* metabolism assessing tissue glutathione levels (Long-Evans rat)	42651318 (1987) Male rats single IP dose of 0, 7, 70 or 350 mg/kg alachlor for sacrifice at 2, 12 or 24 hours; Male rats single oral doses of 0, 42, 126 or 350 mg/kg alachlor, sacrificed 2 hours postdosing. Single females were also given repeated oral dose of either 350 or 700 mg/kg/day for 14 days and sacrificed at 2 hours postdose, and 2 females/dose administered repeated oral dose of 350 or 700 mg/kg for 13 days and sacrificed at 24 hours postdose. Another group of male rats received alachlor in diet at 0, 0.5, 14, 42 or 126 mg/kg/day for 40 days. Acceptable/nonguideline	GSH depletion observed in liver at 2 hours postdose at 250 mg/kg but recovered 24 hours later. GSH depletion at 12 and 24 hours in nasal mucosa at 70 mg/kg. No decrease in GSH levels seen at dietary levels up to 126 mg/kg/day.
n/a	*In vitro* assay of glutathione levels in rat hepatocytes	43641603 (1995) Incubation of cultured rat hepatocytes for 2 hours with alachlor at concentrations of 0 to 1000 µM. Acceptable/nonguideline	Decreased liver cell GSH levels observed at 300µM and above, 100% depletion at 600 µM and above. Increased LDH leakage and decreased neutral red uptake seen at 400 µM and above. Hepatotoxicity and DNA damaging effect of alachlor may be related to GSH depletion.
n/a	*In vivo* acute exposure to alachlor and hepatotoxicity (F344 rat)	43504101 (1994) 5 rats/dose administered alachlor at 50, 200, 500 or 1000 mg/kg by gavage, with sacrifice at 12 hours postdose. Acceptable/nonguideline	At all dose levels GSH levels decreased by 10-56% over the dose range tested. At 500 mg/kg and higher, serum ALT, AST and LDH were elevated and microscopic lesions (centrilobular cytoplasmic eosinophilia, inflammation and degeneration/necrosis). UDS response may reflect cytotoxicity.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
n/a	*In vivo* whole body autoradiography (Long-Evans rat, CD-1 mouse, squirrel monkey)	42852103 (1985) Female rats, mice and male monkeys given gavage doses of radiolabeled alachlor at 7, 70 or 700 mg/kg and female rats given single dermal doses of 7 or 700 mg/kg; sacrifice at 24 or 120 hours postdosing. Acceptable/nonguideline	At 24 hours, blood, liver, kidney, nasal vibrissae, body hair, mouth surfaces, teeth and roots surfaces and periorbital fat of all species labeled. At 120 hours, only blood in rat and mouse labeled; overall labeling less in monkey than rat or mouse, significant nasal turbinate labeling in rat with less in mouse, absent in monkey. No change in distribution associated with dose.
n/a	*In vivo* whole body autoradiography (Long-Evans rat)	42852104 (1989) Male and female rats administered gavage doses of 7 or 700 mg/kg alachlor, metolachlor or MON 4601. Sacrifice at 24 and 120 hours postdose Acceptable/nonguideline	At 24 hours distribution was similar for all compounds with more lung and nasal distribution for alachlor and MON 4601 than metolachlor. At 120 hours, radioactivity still localized in intestines, liver, kidney, heart and lungs for all chemicals, with more nasal turbinate labeling for metolachlor and alachlor than MON 4601 at 7 mg/kg and less for alachlor than metolachlor or MON 4601 at 700 mg/kg; otherwise, high dose at 120 hours was similar except for more localization over the stomach than low dose. Data indicate faster clearance of alachlor from intestinal tract and that metolachlor and MON 4601 undergo biliary excretion/enterohepatic circulation.
n/a	*In vivo* whole body autoradiography (Long-Evans rat)	42651304 (1988) Female rats received single gavage doses of 0.7 or 7.0 mg/kg radiolabeled alachlor methylsulfide. Sacrifice at 24 and 120 hours postdose Acceptable/nonguideline	At 24 hours localization observed in the intestines, stomach, nasal turbinates; lesser amounts in liver, kidney, stomach lining, mouth lining, eye orbit and harderian gland. At 120 hours, nasal turbinate localization evident.
n/a	*In vivo* whole body autoradiography (Sprague-Dawley rat, CD-1 mouse)	43507401 (1993) Female rats and mice given 7 or 70 mg/kg radiolabeled DEA, sacrificed at 24 hours Acceptable/nonguideline	Localization in rats observed in highly perfused tissues (liver, lungs, kidney outer cortex, heart) at both doses with intense localization also seen in nasal mucosa and tongue lining. Localization in mice seen in liver, lungs, intestines (fecal material), stomach contents and stomach lining, heart and kidneys, but not over nasal mucosa.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
n/a	*In vivo* whole body autoradiography (Sprague-Dawley rat, CD-1 mouse)	43706001 (1991) Male and female rats and mice given 7 or 70 mg/kg single oral dose of dimethylaniline (DMA); sacrifice at 24 and 120 hours postdosing Acceptable/nonguideline	In the rat, localization was seen in gut contents, nasal mucosa and liver at 24 hours (similar but more intense at 70 than 7 mg/kg); at 120 hours, localization seen in nasal mucosa, also liver, kidney, heart, blood and lungs. In mice, localization was seen in liver, gut contents and stomach lining at 24 hours and only slight labeling in liver and stomach lining at 120 hours.
n/a	*In vivo* whole body autoradiography (Sprague-Dawley, Fisher 344 and Long-Evans rats; golden Syrian hamster)	42852105 (1992) Animals given 7 or 70 mg/kg single gavage dose of radiolabeled alachlor; sacrifice at 24 or 120 hours postdose Acceptable/nonguideline	In rat at 24 hours, similar distribution seen in all rats (liver, lungs, heart, kidney, adrenal gland, spleen, intestinal contents, nasal mucosa), with nasal staining most pronounced in Long-Evans rats. At 120 hours, liver, kidney, adrenal, heart and lungs major sites. In hamster, at 24 hours localization seen in feces, stomach contents, bladder and liver, with liver major area at 120 hours postdose.
n/a	*In vivo* DNA binding study (Long-Evans rat)	43369201 (1994) Single oral gavage dose of 125 mg/kg unlabeled:labeled (28:1) alachlor with sacrifice at 24 hours Unacceptable/nonguideline	DNA binding study had numerous uncertainties relating to administered dose, method sensitivity and protein contamination of DNA samples. However, the was qualitative evidence that radioactivity was covalently bound to DNA in nasal tissues.
n/a	*In vivo* cellular stress gene response study (Long-Evans rats)	43590002 (1995) 126 mg/kg alachlor in diet for 30 or 60 days Unacceptable/nonguideline	PCR analysis demonstrated that (1) HSP 70 was induced only in olfactory epithelia at 60 days (2.1-fold) and NQO1 was induced in both olfactory (3.4-fold) and respiratory (2.2-fold) epithelia.
n/a	*In vivo* cell proliferation study (rat and mouse)	42852102 (1991) Administered in diet for up to 60 days ranging from 0.5 to 260 mg/kg/day Acceptable/nonguideline	Cell proliferation as measured by ^{3}H-thymidine incorporation seen at 126 and 260 mg/kg/day in nasal olfactory epithelium of rats but not mice. Reversible response. NOAEL for cell proliferation in rat nasal turbinates was 46 mg/kg/day in this study.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
n/a	Covalent adduct formation *in vivo* in nasal tissue protein (Long-Evans rat)	43641604 (1995) Female rats fed radiolabeled alachlor in diet at 126 mg/kg/day for 13 days, with sacrifice at days 1, 3, 7 and 13. Acceptable/nonguideline	Direct correlation observed between amount of alachlor binding to rat nasal proteins and duration of exposure. Major adduct identified was 3,5-diethylbenzoquinone-4-imine (DEBQI)-cysteine.
n/a	*In vitro* cytotoxicity (Long-Evans rat)	43641602 (1995) Alachlor and its metabolites, DEA, secondary sulfide and secondary amide were assayed in preparations of rat nasal turbinate tissue at 1 or 5 mM *in vitro* Acceptable/nonguideline	Cytotoxicity as measured by acid phosphatase release into culture medium was observed at 1 and 5 mM alachlor and at 5 mM DEA but not at 1 mM secondary sulfide or secondary amide (5 mM not tested due to insolubility).
n/a	*In vivo* gastric tumor initiation/promotion study (Sprague-Dawley rat)	43729502 (1994) **Butachlor** administered at 1000 or 3000 ppm (90 or 270 mg/kg/day) in diet or catechol in diet at 8000 ppm for one year, following a single dose of known gastric tumor initiator MNNG. Control groups had single dose of DMSO followed by test diet. Acceptable/nonguideline	Butachlor did not show initiating potential on its own when used at dose levels which produced gastric tumors in the chronic rat study. It did enhance formation of gastric tumors in combination with the initiating agent MNNG.
n/a	*In vivo* dietary study on effect of impurities on gastric tumor formation (Sprague-Dawley rat)	43750801 (1995) **Butachlor** technical administered at 0, 100, 1000 or 3000 ppm (0, 5, 50 or 150 mg/kg/day) in diet to female rats for up to 22 months with interim sacrifices at numerous times; another group of female rats administered analytical grade butachlor at 3000 ppm for 22 months Acceptable/nonguideline	At 300 ppm cell proliferation of fundus increased at most study intervals, reversible between days 30-64. Mucosal thickness decreased throughout study, gastric pH and serum gastrin increased at high dose, with reduced glutathione in stomach tissue observed. Induction of stomach tumors by butachlor not due to impurities (40/171 at 3000 ppm technical and 20/60 at 3000 ppm analytical. Tumors characterized as neuroendocrine. Thyroid parameter measurements showed disruption of thyroid-pituitary homeostasis initiated by increased UDPGT activity. PCNA staining of nasal olfactory epithelium observed.

Guideline No.	Study Type	MRID No. (year)/ Classification /Doses	Results
n/a	*In vivo* cell proliferation study (CD-1 mouse)	43729503 (1994) **Butachlor** in diet at 0 or 2000 ppm (0 or 300 mg/kg/day) for 14 and 60 days in female mice Acceptable/nonguideline	No consistent increase in cell proliferation was reported in either the fundic or pyloric regions. A slight increase in the fundic neck region but not in base region was seen and there was no evidence of toxicity in the mucosa.
n/a	*In vivo* gastric cell proliferation and mucosal thickness study (Rhesus monkey)	43729501 (1994) **Butachlor** by gavage single dose at 0, 100, 200 or 400 mg/kg in female monkeys Acceptable/nonguideline	No changes in cell proliferation or mucosal thickness were reported.
n/a	*In vivo* gastric tumor promotion study (Long-Evans rat)	43590001 (1995) Alachlor administered in diet at 15 or 126 mg/kg/day for 1 year (other groups received control diet or 8000 ppm catechol) following a single dose of the known gastric tumor initiator MNNG. Noninitiated groups received 5 mL/kg DMSO followed by dietary alachlor at 126 mg/kg/day. Acceptable/nonguideline	Alachlor promoted tumor formation in the glandular stomach in females, less in males. Alachlor alone produced no tumors in males and 4 tumors in females. With MNNG initiation, 75% of females and 30% of males showed tumors at 126 mg/kg/day in glandular stomach fundus. MNNG alone caused only 1 tumor. Alachlor produced atrophy of fundic mucosa at 126 mg/kg/day with or without MNNG and reduced stomach fluid and gastric HCl secretion, with increased serum gastrin and increased stomach pH. Study authors concluded that data provided evidence that alachlor produced glandular stomach tumors in rats by the same mechanism as butachlor.