2,4-Dichlorophenoxyacetic acid
related to birth defects & other abnormal development, incl. epidemiology;
mutagen*, clastogen*, genotox*
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Pochettino AA, Bongiovanni B, Duffard RO, Evangelista de Duffard AM. 2011 Mar 3. Oxidative stress in ventral prostate, ovary, and breast by 2,4-dichlorophenoxyacetic acid in pre- and postnatal exposed rats. Environ Toxicol .[1 SEP]Abstract: The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has been widely used in agriculture and forestry since the 1940s. 2,4-D has been shown to produce a wide range of adverse effects-from embryotoxicity and teratogenicity to neurotoxicity-on animal and human health. The purpose of this study was to determine the possible effects of pre- and postnatal exposure to 2,4-D on oxidative stress in ventral prostate, ovary and breast. Pregnant rats were daily exposed to oral doses of 70 mg/kg/day of 2,4-D from 16 days of gestation up to 23 days after delivery. Then, the pups were sacrificed by decapitation at postnatal day (PND) 45, 60, or 90. Antioxidant enzyme activities and some parameters of the oxidative stress were assessed in ventral prostate, breast, and ovary. Results show that 2,4-D produced three different effects. First, it increased the concentration of some radical oxygen species and the rates of lipid peroxidation and protein oxidation in ventral prostate, thereby causing oxidative stress at all ages studied. Although an increase in the activity of some antioxidant enzymes was detected, this seemed to have been not enough to counteract the oxidative stress. Second, 2,4-D promoted the oxidative stress in the breasts, mainly during puberty and adulthood, probably because the developing gland is more sensitive to xenobiotics than the adult organ. Third, 2,4-D altered the activity of some antioxidant enzymes and increased lipid peroxide concentration in the ovary. This effect could reflect the variety of ovarian cell types and their different responses to endocrine changes during development. (c) 2011 Wiley Periodicals, Inc. Environ Toxicol, 2011.

Sandal S, Yilmaz B. 2010 Mar 1. Genotoxic effects of chlorpyrifos, cypermethrin, endosulfan and 2,4-D on human peripheral lymphocytes cultured from smokers and nonsmokers. Environ Toxicol .[1 SEP]Abstract: Pesticides often cause environmental pollution and adverse effects on human health. We have chosen four structurally different pesticides (endosulfan, an organochlorine pesticide; chlorpyrifos, an organophosphate insecticide; cypermethrin, type II pyrethroid insecticide, and 2,4-dichlorophenoxyacetic acid, a chlorinated aromatic hydrocarbon acid pesticide) to examine and compare their effects on DNA damage in acutely cultured human lymphocytes by the comet assay. In addition, possible differences in response between smoking and nonsmoking subjects were also investigated. Venous blood samples were obtained from healthy male nonsmoker (n = 7) and smoker (n = 8) donors. Primary cultures of lymphocytes were prepared and test groups were treated with three different concentrations (1, 5, and 10 muM) of endosulfan, chlorpyrifos, cypermehrin, and 2,4-D. DNA damage was assessed by alkaline comet assay. We determined an increase in the ratio of DNA migration in human lymphocyte cell cultures as a result of treatment with cypermethrin, 2,4-D and chlorpyrifos at high concentration. Endosulfan had no significant genotoxic effect even at 10 muM concentration. We suggest that chlorpyrifos and cypermethrin are more potentially genotoxic than endosulfan and 2,4-D. Our findings also indicate that the only significant DNA damage between smokers and nonsmokers was observed in the 2,4-D-treated group. (c) 2010 Wiley Periodicals, Inc. Environ Toxicol, 2010.

Ngo AD, Taylor R, Roberts CL. 2010. Paternal exposure to Agent Orange and spina bifida: a meta-analysis. Eur J Epidemiol 25(1): 37-44.[1 SEP]Abstract: The objective of this study is to conduct a meta-analysis of published and unpublished studies that examine the association between Agent Orange (AO) exposure and the risk of spina bifida. Relevant studies were identified through a computerized literature search of Medline and Embase from 1966 to 2008; a review of the reference list of retrieved articles and conference proceedings; and by contacting researchers for unpublished studies. Both fixed-effects and random-effects models were used to pool the results of individual studies. The Cochrane Q test and index of heterogeneity (I(2)) were used to evaluate heterogeneity, and a funnel plot and Egger's test were used to evaluate publication bias. Seven studies, including two Vietnamese and five non-Vietnamese studies, involving 330 cases and 134,884 non-cases were included in the meta-analysis. The overall relative risk (RR) for spina bifida associated with paternal exposure to AO was 2.02 (95% confidence interval [CI]: 1.48-2.74), with no statistical evidence of heterogeneity across studies. Non-Vietnamese studies showed a slightly higher summary RR (RR = 2.22; 95% CI: 1.38-3.56) than Vietnamese studies (RR = 1.92 95% CI: 1.29-2.86).

When analyzed separately, the overall association was statistically significant for the three case-control studies (Summary Odds Ratio = 2.25, 95% CI: 1.31-3.86) and the cross sectional study (RR = 1.97, 95% CI: 1.31-2.96), but not for the three cohort studies (RR: 2.11; 95% CI: 0.78-5.73). Paternal exposure to AO appears to be associated with a statistically increased risk of spina bifida.

Fraser FC. 2009 May. Does paternal exposure to Agent Orange cause birth defects? Am J Med Genet A 149A(5):835-6.

Konjuh C, Garcia G, Lopez L, de Duffard AM, Brusco A, Duffard R. 2008 Aug. Neonatal hypomyelination by the herbicide 2,4-dichlorophenoxyacetic acid. Chemical and ultrastructural studies in rats. Toxicol Sci 104(2):332-40.Abstract: The purpose of this study was to determine whether 2,4-dichlorophenoxyacetic acid (2,4-D), which is an herbicide used to control the growth of broadleaf weeds, had a direct or an indirect (mediated by undernutrition) hypomyelinating effect. We also proposed to analyze the effect of 2,4-D on undernourished (UN) pups. Four experimental rat groups were used: well-nourished (WN) pups, litters with eight offsprings; UN pups, litters with fourteen offsprings; WN pups whose mother received 70 mg/kg/day of 2,4-D from postnatal day (PND) 9 to 21 (WN70 pups); and UN pups whose mother received 70 mg/kg/day of 2,4-D from PND 9 to 21 (UN70 pups). In this work, we demonstrated that (1) myelin proteins (analyzed by Western blot and/or immunohistochemical study) showed a significant decrease in WN70, UN, and UN70 with respect to control group; (2) there is a good correlation between these myelin-specific protein expression with the degree of myelin compaction detected by electron microscopy in groups exposed to 2,4-D; (3) a decreased and normal number of myelin sheets were detected in UN and 2,4-D exposed pups, respectively; and (4) undernourishment sensitized pups to the hypomyelinating effect of 2,4-D. According to this and besides the fact that WN70 group have no body weight changes, these results are indicating that 2,4-D and undernourishment are two independent hypomyelinating factors.

Sturtz N, Deis RP, Jahn GA, Duffard R, Evangelista de Duffard AM. 2008 May 21. Effect of 2,4-dichlorophenoxyacetic acid on rat maternal behavior. Toxicology 247(2-3):73-9.Abstract: Exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) has several deleterious effects on the nervous system such as alterations in the concentrations of neurotransmitters in the brain and/or behavioral changes, myelination rate, ganglioside pattern [Bortolozzi, A., Duffard, R., Antonelli, M., Evangelista de Duffard, A.M., 2002. Increased sensitivity in dopamine D(2)-like brain receptors from 2,4-dichlorophenoxyacetic acid (2,4-D)-exposed and amphetamine-challenged rats. Ann. N.Y. Acad. Sci. 965, 314-323; Duffard, R., Garcia, G., Rosso, S., Bortolozzi, A., Madariaga, M., DiPaolo, O., Evangelista de Duffard, A.M., 1996. Central nervous system myelin deficit in rats exposed to 2,4-dichlorophenoxyacetic acid throughout lactation. Neurotoxicol. Teratol. 18, 691-696; Evangelista de Duffard, A.M., Orta, C., Duffard, R., 1990. Behavioral changes in rats fed a diet containing 2,4-dichlorophenoxyacetic butyl ester. Neurotoxicology 11, 563-572; Evangelista de Duffard, A.M., Bortolozzi, A., Duffard, R.O., 1995. Altered behavioral responses in 2,4-dichlorophenoxyacetic acid treated and amphetamine challenged rats. Neurotoxicology 16, 479-488; Munro, I.C., Carlo, G.L., Orr, J.C., Sund, K., Wilson, R.M. Kennepohl, E. Lynch, B., Jablinske, M., Lee, N., 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. J. Am. Coll. Toxicol. 11, 559-664; Rosso et al., 2000], and its administration to pregnant and lactating rats adversely affects litter growth and milk quality. Since normal growth of the offspring depends on adequate maternal nursing and care, we evaluated the effect of 2,4-D on rat maternal behavior as well as the dam's monoamine levels in arcuate nucleus (AcN) and serum prolactin (PRL) levels. Wistar dams were exposed to the herbicide through the food from post partum day (PPD) 1 to PPD 7. Dams were fed either with a 2,4-D treated diet (15, 25 or 50mg 2,4-D/kg/daybw) or with a control diet. We observed that maternal nesting behavior was not modified by 2,4-D treatment. However, mother-pup interactions, specially the nursing behavior, were altered. Retrieval, crouching and licking of pups were reduced or suspended after 2,4-D treatment. We also observed an increase in the latency of retrieval and crouching in the dams treated with the herbicide. Dams showed movement along cage peripheries, food consumption during the light phase and high self-grooming. In addition of the deficits observed in maternal behavior parameters, increased catecholamine levels and a drastic decrease in indolamine levels in the AcN of treated dams were determined. Serum PRL levels were also diminished by 62%, 68% and 70% with respect to control dams in the 15, 25 and 50mg 2,4-D/kgbw treated dams, respectively. In conclusion, exposure to 2,4-D during the first post partum days produced changes in maternal behavior, serum prolactin and monoamine levels in the AcN of treated dams.

Roman GC. 2007 Nov 15. Autism: transient in utero hypothyroxinemia related to maternal flavonoid ingestion during pregnancy and to other environmental antithyroid agents. J Neurol Sci 262(1-2):15-26. Abstract: The incidence and prevalence of autism have increased during the past two decades. Despite comprehensive genetic studies the cause of autism remains unknown. This review emphasizes the potential importance of environmental factors in its causation. Alterations of cortical neuronal migration and cerebellar Purkinje cells have been observed in autism. Neuronal migration, via reelin regulation, requires triiodothyronine (T3) produced by deiodination of thyroxine (T4) by fetal brain deiodinases. Experimental animal models have shown that transient intrauterine deficits of thyroid hormones (as brief as 3 days) result in permanent alterations of cerebral cortical architecture reminiscent of those observed in brains of patients with autism. I postulate that early maternal hypothyroxinemia resulting in low T3 in the fetal brain during the period of neuronal cell migration (weeks 8-12 of pregnancy) may produce morphological brain changes leading to autism. Insufficient dietary iodine intake and a number of environmental antithyroid and goitrogenic agents can affect maternal thyroid function during pregnancy. The most common causes could include inhibition of deiodinases D2 or D3 from maternal ingestion of dietary flavonoids or from antithyroid environmental contaminants. Some plant isoflavonoids have profound effects on thyroid hormones and on the hypothalamus-pituitary axis. Genistein and daidzein from soy (Glycine max) inhibit thyroperoxidase that catalyzes iodination and thyroid hormone biosynthesis. Other plants with hypothyroid effects include pearl millet (Pennisetum glaucum) and fonio millet (Digitaria exilis); thiocyanate is found in Brassicae plants including cabbage, cauliflower, kale, rutabaga, and kohlrabi, as well as in tropical plants such as cassava, lima beans, linseed, bamboo shoots, and sweet potatoes. Tobacco smoke is also a source of thiocyanate. Environmental contaminants interfere with thyroid function including 60% of all herbicides, in particular 2,4-dichlorophenoxyacetic acid (2,4-D), acetochlor, aminotriazole, amitrole, bromoxynil, pendamethalin, mancozeb, and thioureas. Other antithyroid agents include polychlorinated biphenyls (PCBs), perchlorates, mercury, and coal derivatives such as resorcinol, phthalates, and anthracenes. A leading ecological study in Texas has correlated higher rates of autism in school districts affected by large environmental releases of mercury from industrial sources. Mercury is a well known antithyroid substance causing inhibition of deiodinases and thyroid peroxidase. The current surge of autism could be related to transient maternal hypothyroxinemia resulting from dietary and/or environmental exposure to antithyroid agents. Additional multidisciplinary epidemiological studies will be required to confirm this environmental hypothesis of autism.

Soloneski S, Gonzalez NV, Reigosa MA, Larramendy ML. 2007 Nov. Herbicide 2,4-dichlorophenoxyacetic acid (2,4-D)-induced cytogenetic damage in human lymphocytes in vitro in presence of erythrocytes. Cell Biol Int 31(11):1316-22. Abstract: The genotoxic effects of 2,4-D and its commercial derivative 2,4-D DMA were studied by measuring sister chromatid exchange (SCE), cell-cycle progression and mitotic index in human whole blood (WBC) and plasma leukocyte cultures (PLC). Concentrations of 10, 25, 50 and 100 microg herbicide/ml were used during 72 h. In WBC, a significant increase in SCE frequency was observed within the 10-50 microg 2,4-D/ml and 25-100 microg 2,4-D DMA/ml dose range. Contrarily, in PLC, none of the concentrations employed affected the SCEs frequency. A significant delay in cell proliferation was observed in WBC after treatments with 25 and 50 microg 2,4-D/ml and 50 and 100 microg 2,4-D DMA/ml. In PLC, only 100.0 microg 2,4-D/ml altered cell-cycle progression. For both chemicals, a progressive dose-related inhibition of mitotic activity was observed. The results demonstrated that the presence of erythrocytes in the culture system modulated the DNA and cellular damage inflicted by 2,4-D and 2,4-D DMA into human lymphocytes in vitro as well as both 2,4-D and 2,4-D DMA were more potent genotoxic agents in the presence of human red cells.

Dinamarca VM, Hidalgo ME, Cavieres MF. 2007 Jul 31. Lack of effects of 2,4-dichlorophenoxyacetic acid administration on markers of oxidative stress during early pregnancy in mice. Toxicology 237(1-3):104-10. Abstract: Induction of oxidative stress by 2,4-dichlorophenoxyacetic acid (2,4-D) both as a pure compound and in commercial formulation was investigated during early pregnancy in mice. Pregnant animals were exposed to increasing doses of the herbicide (0.01, 0.1 and 100mg/kg/d) during gestation days 0-9, after which animals were euthanized and their blood analyzed for catalase activity, thiobarbituric acid reactive substances (TBARs) and total antioxidant capacity (TAC). Number of corpora lutea and uterine implantations and resorptions were also determined. Herbicide exposure did not cause any overt signs of maternal toxicity at any of the doses administered; neither did it cause an effect on developmental parameters. Catalase activity and TBARs were not modified by herbicide exposure although TAC was

significantly decreased at 100mg/kg/d of both pure and formulated compound. Thus, 2,4-D does not seem to induce oxidative stress during early pregnancy in mice at the doses administered, indicating that this mechanism is probably not involved in mediating herbicide toxicity at these dose levels. Furthermore, since no manifestations of developmental toxicity were observed after administration of the herbicide, it is also possible that 2,4-D may not produce any early developmental toxicity at the low environmentally relevant doses tested in this animal model.

Maire MA, Rast C, Landkocz Y, Vasseur P. 2007 Jul 28. 2,4-Dichlorophenoxyacetic acid: Effects on Syrian hamster embryo (SHE) cell transformation, c-Myc expression, DNA damage and apoptosis. Mutat Res 631(2):124-36. Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D) is a selective, systemic auxin-type herbicide extensively used throughout the world. The present research was aimed at studying effects of low and non-cytotoxic concentrations of 2,4-D on SHE cells in relation with carcinogenicity. Effects were studied on Syrian hamster morphological cell transformation, c-Myc expression - both at the gene and protein level - DNA damage and apoptosis. 2,4-D significantly induced cell transformation at 11.5muM and 23muM (i.e. 2.5mug/mL and 5mug/mL). An increase in the expression of the transcription factor c-Myc, measured by use of RT-PCR with respect to mRNA level and by Western blotting for protein level was registered at these concentrations, as well as genotoxic effects evaluated with the single-cell gel electrophoresis (Comet) assay. Consequences for apoptosis of 2,4-D treatment were also investigated. The fluorochrome acridine orange was used to study DNA fragmentation as a marker of apoptosis. No effect on apoptosis was found at 2,4-D concentrations that induced cell transformation. This was confirmed by the unchanged expression of Bcl-2 and Bax, two regulator genes of the mitochondrial pathway of apoptosis. Our results demonstrate the transforming and genotoxic effects of low concentrations of 2,4-D in mammalian cells. This information contributes to a better understanding of the mechanism of 2,4-D toxicity in mammalian cells and demonstrates that 2,4-D should be considered as potentially hazardous to humans.

Rowland RE, Edwards LA, Podd JV. 2007. Elevated sister chromatid exchange frequencies in New Zealand Vietnam War veterans. Cytogenet Genome Res 116(4):248-51. Abstract: From July 1965 until November 1971, New Zealand Defence Force Personnel fought in the Vietnam War. During this time more than 76,500,000 litres of phenoxylic herbicides were sprayed over parts of Southern Vietnam and Laos, the most common being known as 'Agent Orange'. The current study aimed to ascertain whether or not New Zealand Vietnam War veterans show evidence of genetic disturbance arising as a consequence of their now confirmed exposure to these defoliants. A sample group of 24 New Zealand Vietnam War veterans and 23 control volunteers were compared using an SCE (sister chromatid exchange) analysis. The results from the SCE study show a highly significant difference ($P < 0.001$) between the mean of the experimental group (11.05) and the mean of a matched control group (8.18). The experimental group also has an exceptionally high proportion of HFCs (cells with high SCE frequencies) above the 95th percentile compared to the controls (11.0 and 0.07%, respectively). We conclude that the New Zealand Vietnam War veterans studied here were exposed to a clastogenic substance(s) which continues to exert an observable genetic effect today, and suggest that this is attributable to their service in Vietnam.

Ferri A, Duffard R, de Duffard AM. 2007. Selective oxidative stress in brain areas of neonate rats exposed to 2,4-Dichlorophenoxyacetic acid through mother's milk. Drug Chem Toxicol 30(1):17-30. Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D) induced disparate alterations on enzymatic activities of the defensive mechanism and/or modifications of the reactive oxygen species levels in specific neonate rat brain regions. The midbrain, striatum, and prefrontal cortex were the areas where the alterations were more remarkable and with similar tendency. The hippocampus did not suffer many alterations, and the hypothalamus was the area where no changes were observed. The current results suggest that the developing brain areas have different susceptibilities to the adverse effect of the herbicide, especially those areas related to the dopaminergic system, and that oxidative stress is one 2,4-D mechanism of toxicity.

Schecter A, Constable JD. 2006 Oct. Commentary: Agent Orange and birth defects in Vietnam. Int J Epidemiol 35(5):1230-2.

Ngo AD, Taylor R, Roberts CL, Nguyen TV. 2006 Oct. Association between Agent Orange and birth defects: systematic review and meta-analysis. Int J Epidemiol 35(5):1220-30. Abstract: BACKGROUND: The association between parental exposure to Agent Orange or dioxin and birth defects is controversial, due to

inconsistent findings in the literature. The principal aim of this study was to conduct a meta-analysis of relevant epidemiological studies that examined this association and to assess the heterogeneity among studies. METHODS: Relevant studies were identified through a computerized literature search of Medline and Embase from 1966 to 2002; reviewing the reference list of retrieved articles and conference proceedings; and contacting researchers for unpublished studies. A specified protocol was followed to extract data on study details and outcomes. Both fixed-effects and random-effects models were used to synthesize the results of individual studies. The Cochrane Q test and index of heterogeneity (I2) were used to evaluate heterogeneity, and a funnel plot and Egger's test were used to evaluate publication bias. RESULTS: In total, 22 studies including 13 Vietnamese and nine non-Vietnamese studies were identified. The summary relative risk (RR) of birth defects associated with exposure to Agent Orange was 1.95 [95% confidence interval (95% CI) 1.59-2.39], with substantial heterogeneity across studies. Vietnamese studies showed a higher summary RR (RR = 3.00; 95% CI 2.19-4.12) than non-Vietnamese studies (RR = 1.29; 95% CI 1.04-1.59). Sub-group analyses found that the magnitude of association tended to increase with greater degrees of exposure to Agent Orange, rated on intensity and duration of exposure and dioxin concentrations measured in affected populations. CONCLUSION: Parental exposure to Agent Orange appears to be associated with an increased risk of birth defects.

Sturtz N, Bongiovanni B, Rassetto M, Ferri A, de Duffard AM, Duffard R. 2006 Jan. Detection of 2,4-dichlorophenoxyacetic acid in rat milk of dams exposed during lactation and milk analysis of their major components. Food Chem Toxicol 44(1):8-16.SEPAbstract: 2,4-Dichlorophenoxyacetic acid (2,4-D) and its derivatives are herbicides widely used to control the growth of broadleaf and woody plant. Human and animal exposure to 2,4-D through agriculture use, food products, or use in lawn and garden care has been well documented, but little information is available on the transfer from serum to milk in exposed dams. In this study, we measured the content of 2,4-D in rat milk from mother exposed to 15, 25, 50 or 7 0mg 2,4-D/kg bw through the diet (4 treated groups, 8 dam each; 1 control group with 8 dams) over a period of 16 days starting on the post-natal day 1 (PND 1). The effect of 2,4-D on milk components was also evaluated. All doses tested caused a decrease in the body weight gain of the pups (4 groups, 64 pups each). It also produce a 30% in the content of total lipids and a changed the content of minor proteins in milk of the treated groups. 2,4-D produces an important decrease in some fatty acids content, being the polyunsaturated fatty acids the most affected. Further analysis showed that 2,4-D concentrations chromatographically detected both serum of dams and pups and milk were dose-dependent.

Garcia GB, Konjuh C, Duffard RO, Evangelista de Duffard AM. 2006. Dopamine-beta-hydroxylase immunohistochemical study in the locus coeruleus of neonate rats exposed to 2,4-dichlorophenoxyacetic acid through mother's milk. Drug Chem Toxicol 29(4):435-42.SEPAbstract: Dopamine-beta-hydroxylase (DbetaH), the enzyme that synthesizes noradrenaline from dopamine, was studied in the locus coeruleus (LC) of neonate rats exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) through lactation for 14 days (from PND 9 to 22). Pups (22 days old) were anesthetized and fixed by transcardiac perfusion. Control and treated serial sections from brain stem--which correspond with the LC according to the Paxinos and Watson atlas--were simultaneously processed by an immunohistochemistry method for the DbetaH detection. Using an image analysis system, the immunostaining optical density (OD) was measured as an estimation of the enzyme content, and an OD significant decrease in the LC of 2,4-D-exposed animals was observed. As tyrosine hydroxylase levels in the LC are regulated by serotonin and in a previous study we demonstrated that this neurotransmitter was increased in 2,4-D-exposed pups, an indirect effect through serotonergic inhibition could be involved in the decreased DbetaH synthesis in the LC of these pups.

Muta Y, Oneda H, Inouye K. 2005 Mar 1. Anomalous pH-dependence of the activity of human matrilysin (matrix metalloproteinase-7) as revealed by nitration and amination of its tyrosine residues. Biochem J 386(Pt 2):263-70.SEPAbstract: Matrilysin activity exhibits a broad bell-shaped pH-dependence profile, with pK(a) values of 4.0 and 9.8. A maximum of five out of eight tyrosine residues in matrilysin were nitrated with tetranitromethane. On nitration of between one and five tyrosines, pK(a) at the alkaline side (pK(e2)) was shifted from 9.8 to 10.3-10.6, while that at the acidic side (pK(e1)) was not altered. The pK(e2) that was shifted by nitration to 10.3-10.6 was restored to 9.4-9.7 by subsequent amination, suggesting that the shift in pK(e2) is induced by a negative charge introduced on the most reactive tyrosine, Tyr-150. The Michaelis constant (K(m)) observed at pH 10 was decreased by nitration as a result of the increase in pK(e2), suggesting that the residue with pK(e2) may play a role in the recognition of substrate. When four or five

tyrosines were nitrated, the activity at pH <7 decreased significantly, while that at pH 7-10 was unchanged, and thus the pH-dependence was not bell-shaped, but anomalous, with a third pK(a) (pK(e3)) of 6.2-6.4 in addition to pK(e1) and pK(e2). This suggests the possibility that a newly introduced nitrotyrosine residue has a strong influence on the activity as an ionizable group.

Razzaghi M, Kodell R. 2004 Dec. Quantitative risk assessment for developmental neurotoxic effects. Risk Anal 24(6):1673-81.Abstract: Developmental neurotoxicity concerns the adverse health effects of exogenous agents acting on neurodevelopment. Because human brain development is a delicate process involving many cellular events, the developing fetus is rather susceptible to compounds that can alter the structure and function of the brain. Today, there is clear evidence that early exposure to many neurotoxicants can severely damage the developing nervous system. Although in recent years, there has been much attention given to model development and risk assessment procedures for developmental toxicants, the area of developmental neurotoxicity has been largely ignored. Here, we consider the problem of risk estimation for developmental neurotoxicants from animal bioassay data. Since most responses from developmental neurotoxicity experiments are nonquantal in nature, an adverse health effect will be defined as a response that occurs with very small probability in unexposed animals. Using a two-stage hierarchical normal dose-response model, upper confidence limits on the excess risk due to a given level of added exposure are derived. Equivalently, the model is used to obtain lower confidence limits on dose for a small negligible level of risk. Our method is based on the asymptotic distribution of the likelihood ratio statistic (cf. Crump, 1995). An example is used to provide further illustration.

Garcia G, Tagliaferro P, Ferri A, Evangelista de Duffard AM, Duffard R, Brusco A. 2004 Dec. Study of tyrosine hydroxylase immunoreactive neurons in neonate rats lactationally exposed to 2,4-dichlorophenoxyacetic Acid. Neurotoxicology 25(6):951-7.Abstract: Dopaminergic neurons from the midbrain nuclei substantia nigra (SN; A9) and ventral tegmental area (VTA; A10) were investigated by tyrosine hydroxylase (TH) immunostaining in neonate rat brains exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) through lactation. Dorsal raphe serotonin (5-HT) projections to SN and VTA were also studied by 5-HT transporter (5-HTT) immunostaining and results were quantified by image analysis. Twenty-five-day-old pups exposed to 2,4-D through mothers milk were used. Dams were intraperitoneally administered 70 or 100mg/kg/day of 2,4-D from the 9th to the 25th postpartum day. After 100mg/kg of 2,4-D exposure, a 25% diminution in the SN and a 33% diminution in the VTA neurons' TH immunostaining along with a significantly 5-HT fiber density diminution were observed. The present work supports previous reports which suggest that exposure to 2,4-D during development has multiple effects on CNS.

Zeljezic D, Garaj-Vrhovac V. 2004 Jul 15. Chromosomal aberrations, micronuclei and nuclear buds induced in human lymphocytes by 2,4-dichlorophenoxyacetic acid pesticide formulation. Toxicology 200(1):39-47.Abstract: Pesticides of worldwide application are used in agriculture in vast amounts each year, of which herbicides are the most prominent class. Phenoxyacetic herbicides constitute one of the largest groups of herbicides sold in the world. Among them, for many years 2,4-dichlorophenoxyacetic acid (2,4-D) has been the one most used. In this study we used Deherban A, a commercial formulation of 2,4-D to determine its possible genotoxic effect on human lymphocytes in vitro by chromosomal aberration analysis and micronucleus assay including the scoring of nuclear buds. Two different concentrations of pesticide formulation were used so that final concentrations of 2,4-D were 0.4 and 4 microg/ml, both in the presence and in the absence of the liver microsomal fraction as metabolic activator. Both concentrations of pesticide caused an increase in chromatid and chromosome breaks, number of micronuclei and number of nuclear buds. Presence of the S9 mix additionally elevated the number of chromatid breaks and micronuclei in treated lymphocytes.

Bortolozzi AA, Evangelista De Duffard AM, Duffard RO, Antonelli MC. 2004 Jul-Aug. Effects of 2,4-dichlorophenoxyacetic acid exposure on dopamine D(2)-like receptors in rat brain. Neurotoxicol Teratol 26(4):599-605.Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D), a worldwide-used herbicide, has been associated with a range of adverse health effects on humans and different animal species. Although the mechanism of 2,4-D neurotoxicity remains unknown, we had previously reported changes in various neurotransmitter systems, such as serotonin (5-HT) and dopamine (DA), which were proposed to mediate some of the behavioral effects in rats. In the present work, we examined the impact of 2,4-D exposure on the ontogeny of dopaminergic D(2)-type receptors in prefrontal cortex (PFc), striatum (CPu), hippocampus

(H) and cerebellum (Cer). Pregnant rats were orally exposed to 70 mg/kg/day of 2,4-D from gestation day (GD) 16 to postpartum day 23. After weaning, the pups were assigned to one of the two subgroups: T1 [fed with untreated diet until postnatal day, (PD) 90] and T2 [maintained with 2,4-D diet until PD 90]. Five to eight pups per age and sex were sacrificed at 6, 15, 30, 45 or 90 days of age for membrane receptor binding assays employing [(3)H]nemonapride. Subchronic 2,4-D exposure (T2 group) increased DA D(2)-type receptor around 40% in CPu. In addition, DA D(2)-type receptor levels also increased in PFc (15 and 30 days) and Cer (30 and 90 days). Sex-dependent differences in D(2) receptors were observed with T2 female rats being more affected than T2 male rats. When the herbicide treatment was interrupted after weaning (T1 group), DA D(2)-type receptor density was apparently recovered and stabilized to control level. These findings suggest a reversible vulnerability of D(2)-type receptors to 2,4-D exposure. Regional increases of D(2)-type receptor density may explain certain behaviors reported early by us, such as catalepsy and right-turning preference in rats exposed to 2,4-D.

Sameshima K, Kobae H, Fofana D, Yoshidome K, Nishi J, Miyata K. 2004 Jun. Effects of pure 2,4-dichlorophenoxyacetic acid on cultured rat embryos. Congenit Anom (Kyoto) 44(2):93-6.Abstract: 2,4-dichlorophenoxyacetic acid (2,4-D), a plant growth regulator, has been used worldwide as a herbicide. Previously we evaluated the prenatal developmental effects of 2,4-D by feeding it to pregnant rats and found that it is maternally toxic and embryolethal, and it induces urogenital malformations in rat fetuses. In the study presented here, we investigated the effects of pure 2,4-D on rat embryos in whole embryo culture. Rat embryos on day 9.5 of gestation were cultured for 48 h at several concentration levels with pure 2,4-D (50-500 microg/mL). 2,4-D caused a concentration-related increase in the incidence of each malformation. Significant decreases in the number of somites were observed at a concentration of 100 microg/mL or more. At the concentration of 100 microg/mL, there was normal yolk sac circulation. This result suggests that 2,4-D has a detrimental effect on somite development and directly damages developing embryos.

Greenlee AR, Ellis TM, Berg RL. 2004 May. Low-dose agrochemicals and lawn-care pesticides induce developmental toxicity in murine preimplantation embryos. Environ Health Perspect 112(6):703-9.Abstract: Occupational exposures to pesticides may increase parental risk of infertility and adverse pregnancy outcomes such as spontaneous abortion, preterm delivery, and congenital anomalies. Less is known about residential use of pesticides and the risks they pose to reproduction and development. In the present study we evaluate environmentally relevant, low-dose exposures to agrochemicals and lawn-care pesticides for their direct effects on mouse preimplantation embryo development, a period corresponding to the first 5-7 days after human conception. Agents tested were those commonly used in the upper midwestern United States, including six herbicides [atrazine, dicamba, metolachlor, 2,4-dichlorophenoxyacetic acid (2,4-D)], pendimethalin, and mecoprop), three insecticides (chlorpyrifos, terbufos, and permethrin), two fungicides (chlorothalonil and mancozeb), a desiccant (diquat), and a fertilizer (ammonium nitrate). Groups of 20-25 embryos were incubated 96 hr in vitro with either individual chemicals or mixtures of chemicals simulating exposures encountered by handling pesticides, inhaling drift, or ingesting contaminated groundwater. Incubating embryos with individual pesticides increased the percentage of apoptosis (cell death) for 11 of 13 chemicals ($p <or= 0.05$) and reduced development to blastocyst and mean cell number per embryo for 3 of 13 agents ($p <or= 0.05$). Mixtures simulating preemergent herbicides, postemergent herbicides, and fungicides increased the percentage of apoptosis in exposed embryos ($p <or= 0.05$). Mixtures simulating groundwater contaminants, insecticide formulation, and lawn-care herbicides reduced development to blastocyst and mean cell number per embryo ($p <or= 0.05$). Our data demonstrate that pesticide-induced injury can occur very early in development, with a variety of agents, and at concentrations assumed to be without adverse health consequences for humans.

Lueken A, Juhl-Strauss U, Krieger G, Witte I . 2004 Feb 28. Synergistic DNA damage by oxidative stress (induced by H2O2) and nongenotoxic environmental chemicals in human fibroblasts. Toxicol Lett 147(1):35-43.Abstract: Genotoxic combination effects of oxidative stress (induced by H2O2) and eight nongenotoxic environmental chemicals (4-chloroaniline, 2,3,4,6-tetrachlorophenol, lindane, 2,4-dichloroacetic acid (2,4-D), m-xylene, glyphosate, nitrilotriacetic acid and n-hexanol) were determined in human fibroblasts. Genotoxicity was measured quantitatively by the single cell gel electrophoresis assay. The nongenotoxic chemicals were used in non cytotoxic concentrations. H2O2 was used in concentrations producing low (50 microM) and no cytotoxicity (40 microM). All environmental chemicals acted in a

synergistic way with H2O2 except DMSO which effectively inhibited H2O(2)-induced DNA damage. The most effective enhancers were 4-chloroaniline, 2,3,4,6-tetrachlorophenol, m-xylene, and n-hexanol. Synergistic effects of hexanol/H2O2 were still evident at a concentration of 0.09 noec (no observed effect concentration). In contrast to synergistic DNA damage in the cell antagonism was found measuring DNA breakage in isolated PM2 DNA. From the results we concluded that synergisms between H2O2 and nongenotoxic chemicals may be a general phenomenon which is not observed on the level of isolated DNA.

Ninomiya K, Nishioka M, Kino-oka M, Taya M. 2004. Differences in responses of plant hairy roots to chemical toxicity compared between primary and ramified roots. Environ Sci 11(5):283-91. Abstract: As a bioassay system for the assessment of the chronic toxicity of chemicals, successive culturing of pak-bung hairy roots was conducted with serial passaging of the primary and lateral root tips. Based on the elongation rates of these root tips, the dose-response profiles were examined while exposing the roots to 2,4-dichlorophenoxyacetic acid (2,4-D) and 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) at concentrations of 0.01-3.0 micromol/dm3, and the values of the median effective concentration, EC50, were determined under the conditions examined. Irrespective of the test chemicals, the EC50 values of both the primary and lateral roots gradually decreased with increasing exposure period ranging from 7 to 35 days. A marked difference in the EC50 values was observed between the primary and lateral roots exposed to 2,4-D; the EC50 value of the lateral roots following exposure for 35 days was 0.036 micromol/dm3, which was one-eighth that of the primary roots for the same exposure period. Moreover, it was found that this phenotypic difference in the root responses was consistently reflected in the distinct patterns of DNA fingerprints of the primary and lateral roots analyzed by the random amplified polymorphic DNA method.

Knopper LD, Lean DRS. 2004. Carcinogenic and genotoxic potential of turf pesticides commonly used on golf courses [review]. J Toxicol Environ Health B Crit Rev 7(4):267-279. Abstract: As a result of the controversy surrounding pesticide use and animal and human health concerns, many municipalities in Canada have restricted, or are in the midst of restricting, the use of pesticides for cosmetic purposes. In some cases, pesticide use on golf courses is also being phased out at the municipal level. One of the dominant health effects of concern in relation to pesticide exposure is the occurrence of cancer. With over 1600 golf courses in Canada and between 400 and 600 new courses created each year in Canada and the United States, there appears to be increasing potential for unintentional human and animal exposure to turf pesticides. In light of the debate around pesticide exposure and the onset of cancer that has lead to controversial Canadian municipal bylaws regulating pesticide use, and due to recent results of a biomonitoring study that has shown genotoxicity in a rodent species living in golf-courses, it seems timely to review the carcinogenic and genotoxic potential of commonly used golf-course pesticides. The purpose of this review is to present some debated epidemiological research that deals with the relationship between pesticide exposure and cancer, and to review and update the literature on the in vivo and in vitro mammalian carcinogenic and genotoxic potential of these pesticides. It is our intention to unite information from various sources so those interested specifically in the carcinogenicity and genotoxicity of pesticides commonly used on golf courses can refer to one comprehensive and updated resource.

Burns CJ, Leonard RC. 2003 Dec. Re: "birth malformations and other adverse perinatal outcomes in four us wheat producing states". Environ Health Perspect 111(16):A869.

Ferri A, Duffard R, Sturtz N, Evangelista de Duffard AM. 2003 Sep-Oct. Iron, zinc and copper levels in brain, serum and liver of neonates exposed to 2,4-dichlorophenoxyacetic acid. Neurotoxicol Teratol 25(5):607-13. Abstract: The effects of 2,4-dichlorophenoxyacetic acid (2,4-D, 70 or 100 mg/kg dam's body weight) on iron (Fe), zinc (Zn) and copper (Cu) in brain, liver and serum of well-nourished and undernourished pups exposed through dam's milk were determined. Undernourishment produced a high Fe decrease (serum and brain) and a delay in weight gain similar to that produced by the highest dose of 2,4-D on well-fed pups. In the latter animals, copper was found to be the most altered ion, increasing its level in serum, liver and some brain areas and decreasing in whole brain. Zinc was the most affected ion in brain areas. Well-nourished pups lactationally exposed to 70 mg 2,4-D/kg dam's body weight altered neither their metal levels nor their body weight in any of the tissues studied. Undernourished pups were more vulnerable to the 2,4-D effect than well-nourished pups. Undernourished pups exposed to a lower 2,4-D dose showed a decrease in their body, brain and liver weight similar to well-fed animals exposed to 100 mg 2,4-D/kg. A noticeable decrease in liver L-tryptophan peroxidase activity by 2,4-D was also registered. This effect was

higher in undernourished and 2,4-D-exposed pups. These results suggest that brain areas have a different susceptibility to the herbicide and that undernourishment produces a higher vulnerability to the herbicide and exacerbates the 2,4-D effect.

Correa-Villasenor A, Cragan J, Kucik J, O'Leary L, Siffel C, Williams L. 2003 Sep. The Metropolitan Atlanta Congenital Defects Program: 35 years of birth defects surveillance at the Centers for Disease Control and Prevention. Birth Defects Res Part A Clin Mol Teratol 67(9):617-24.Abstract: BACKGROUND: The Metropolitan Atlanta Congenital Defects Program (MACDP) is a population-based birth defects surveillance program administered by the Centers for Disease Control and Prevention (CDC) that has been collecting, analyzing, and interpreting birth defects surveillance data since 1967. This paper presents an overview of MACDP current methods and accomplishments over the past 35 years. METHODS: MACDP actively monitors major birth defects among infants born to residents of five counties of metropolitan Atlanta, an area with approximately 50,000 annual births. Cases are ascertained from multiple sources, coded using a modified British Pediatric Association six-digit code, and reviewed and classified by clinical geneticists. RESULTS: MACDP has monitored trends in birth defects rates and has served as a case registry for descriptive, risk factor, and prognostic studies of birth defects, including studies of Agent Orange exposure among Vietnam War veterans, maternal use of multivitamins, diabetes, febrile illnesses, and survival of children with neural tube defects. MACDP has served as a data source for one of the centers participating in the National Birth Defects Prevention Study, and for developing and evaluating neural tube defects prevention strategies related to the periconceptional use of folic acid supplements. CONCLUSIONS: Since its inception, MACDP has served as a resource for the development of uniform methods and approaches to birth defect surveillance across the United States and in many other countries, monitoring birth defects rates, and as a case registry for various descriptive, etiologic, and survival studies of birth defects. MACDP has also served as a training ground for a large number of professionals active in birth defects epidemiology.

Schreinemachers DM. 2003 Jul. Birth malformations and other adverse perinatal outcomes in four U.S. wheat-producing states. Environ Health Perspect 111(9):1259-64.Abstract: Chlorophenoxy herbicides are widely used in the United States and Western Europe for broadleaf weed control in grain farming and park maintenance. Most of the spring and durum wheat produced in the United States is grown in Minnesota, Montana, North Dakota, and South Dakota, with more than 85% of the acreage treated with chlorophenoxy herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA). Rates of adverse birth outcomes in rural, agricultural counties of these states during 1995-1997 were studied by comparing counties with a high proportion of wheat acreage and those with a lower proportion. Information routinely collected and made available by federal agencies was used for this ecologic study. Significant increases in birth malformations were observed for the circulatory/respiratory category for combined sexes [odds ratio (OR) = 1.65; 95% confidence interval (CI), 1.07-2.55]. A stronger effect was observed for the subcategory, which excluded heart malformations (OR = 2.03; 95% CI, 1.14-3.59). In addition, infants conceived during April-June--the time of herbicide application--had an increased chance of being diagnosed with circulatory/respiratory (excluding heart) malformations compared with births conceived during other months of the year (OR = 1.75; 95% CI, 1.09-2.80). Musculoskeletal/integumental anomalies increased for combined sexes in the high-wheat counties (OR = 1.50; 95% CI, 1.06-2.12). Infant death from congenital anomalies significantly increased in high-wheat counties for males (OR = 2.66; 95% CI, 1.52-4.65) but not for females (OR = 0.48; 95% CI, 0.20-1.15). These results are especially of concern because of widespread use of chlorophenoxy herbicides.

Tuschl H, Schwab C. 2003 Mar. Cytotoxic effects of the herbicide 2,4-dichlorophenoxyacetic acid in HepG2 cells. Food Chem Toxicol 41(3):385-93.Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D) and its derivatives are herbicides widely used to control the growth of broadleaf and woody plants. Although 2,4-D is well known to be moderately toxic, little information is available on the mechanisms of its toxicity. Results on carcinogenicity, genotoxicity and mutagenicity are contradictory, but neurotoxic, immunosuppressive and hepatotoxic effects have been defined. The aim of the present study was to investigate the cytotoxic effects of 2,4-D on a human hepatoma cell line. HepG2 cells were treated with different concentrations of 2,4-D, and cell viability, induction of apoptosis/necrosis and cell cycle phases were determined. Apoptosis was detected in flow cytometric light scatter histograms, the annexin V assay, the determination of DNA strand breaks with the TUNEL assay and the occurrence of a sub G(0) peak after propidium iodide (PI) staining.

The induction of apoptosis by 2,4-D was accompanied by a disruption of the mitochondrial membrane potential as verified by staining with the cationic JC-1 probe. In addition, 2,4-D affected the cell cycle in a concentration-dependent manner. Our investigation suggested that 2,4-D exerts its cytotoxic effects by the induction of apoptosis via a direct effect on the mitochondrial membrane potential.

Bortolozzi A, Duffard R, de Duffard AM. 2003 Jan. Asymmetrical development of the monoamine systems in 2,4-dichlorophenoxyacetic acid treated rats. Neurotoxicology 24(1): 149-57.Abstract: The purpose of this study was to determine whether the regional brain biogenic amine levels in adult rats were altered by pre- and post-natal exposure to 2,4-dichlorophenoxyacetic acid (2,4-D). Pregnant rats were daily orally exposed to 70 mg/kg per day of 2,4-D from gestation day (GD) 16 to post-partum day (PPD) 23. After weaning, the pups were assigned to one of two subgroups: T1 fed with untreated diet up to post-natal day (PND) 90 and T2 (maintained with 2,4-D diet up to PND 90). In addition, we wanted to know the effect of 2,4-D on lateralization in the monoamine systems of the basal ganglia of these adult rats and whether there was any correlation with the behavioral developmental pattern previously reported by us. In this study the content of noradrenaline (NA) was significantly increased in substantia nigra (SN) while it decreased in cerebellum in male and female rats of T2 group. The decreased dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovallinic acid (HVA) contents in cerebellum, midbrain, ventral tegmental area (VTA) and prefrontal cortex (PFc) showed an alteration in the mesocorticolimbic system. However, an increase of DA in SN and of DOPAC and HVA in nucleus accumbens (NAc) in both sexes and of DA and DOPAC (only in females) in striatum was detected. The contents of serotonin (5-hydroxytryptamine, 5-HT) were significantly increased in both sexes in PFc, striatum (St), midbrain, SN and cerebellum. Variations of any monoamine levels in NAc and VTA were determined. T1 rats were irreversibly altered: a diminution in DA and/or DOPAC levels in PFc, midbrain, VTA and cerebellum was determined. Indolamines of these rats were increased in both sexes in PFc and St. There was also a large increase in 5-HT levels in midbrain of male rats. Although no changes in the dopaminergic system with respect to their control values in any side of these brain structures were observed, DA and DOPAC levels were found to be decreased in the right side with respect to the left side in striata and accumbens nuclei in T2 female rats supporting the behavioral rotation previously registered by us in these rats. In addition, the increased 5-HT content detected in both the right and left striata observed in this study could be the answer to the behaviors observed and to the early alterations in dopamine in basal ganglia by 2,4-D in neonatal exposed rats, mediated by a serotonergic modulation on the dopaminergic system.

Knapp GW, Setzer RW, Fuscoe JC. 2003. Quantitation of aberrant interlocus T-cell receptor rearrangements in mouse thymocytes and the effect of the herbicide 2,4-dichlorophenoxyacetic acid. Environ Mol Mutagen 42(1):37-43.Abstract: Small studies in human populations have suggested a correlation between the frequency of errors in antigen receptor gene assembly and lymphoid malignancy risk. In particular, agricultural workers exposed to pesticides have both an increased risk for lymphoma and an increased frequency of errors in antigen receptor gene assembly. In order to further investigate the potential of such errors to serve as a mechanistically based biomarker of lymphoid cancer risk, we have developed a sensitive PCR assay for quantifying errors of V(D)J recombination in the thymocytes of mice. This assay measures interlocus rearrangements between two T-cell receptor loci, V-gamma and J-beta, located on chromosomes 13 and 6, respectively. The baseline frequency in four strains of mice was determined at several ages (2-8 weeks of age) and was found to be stable at approximately 1.5 x 10(-5) per thymocyte. Strain AKR, which has a high susceptibility to T-cell lymphomas, did not show an elevated frequency of aberrant V(D)J events. We used this assay to examine the effects of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) on the frequency of these events. Female B6C3F1 mice, 27 days of age, were exposed to 2,4-D by gavage at doses of 0, 3, 10, 30, and 100 mg/kg/day for 4 successive days and sacrificed on day 5. Thymus DNA was isolated and examined for illegitimate V(D)J recombination-mediated gene rearrangements. In addition, pregnant mice were exposed to 2,4-D and thymocytes from the offspring examined at 2 weeks of age. No significant increase in aberrant V(D)J rearrangements was found, indicating that under these conditions 2,4-D does not appear to effect this important mechanism of carcinogenesis.

Holland NT, Duramad P, Rothman N, Figgs LW, Blair A, Hubbard A, Smith MT. 2002 Nov 26. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid in vitro and in vivo. Mutat Res 521(1-2):165-78.Abstract: Widespread use of the herbicide 2,4-

dichlorophenoxyacetic acid (2,4-D) and its association with non-Hodgkin's lymphoma (NHL) and other cancers has raised public concern. Here, micronucleus (MN) formation has been used as a biomarker of genotoxicity, and replicative and mitotic indices (MIs) as biomarkers of cell cycle kinetics in human lymphocytes. Cells were cultured either as whole blood or isolated lymphocytes and treated with pure or commercial forms of 2,4-D at doses between 0.001 and 1 mM for 48 h. Exposure to 2,4-D produced a minimal increase in MN in whole blood and even smaller one in isolated lymphocyte cultures. This induction took place only at levels approaching cytotoxicity and was accompanied by a significant inhibition of replicative index (RI). At a low (0.005 mM) dose of commercial 2,4-D, a small, marginally significant increase in RI (12-15%) was found in two independent sets of experiments (P=0.052). Additionally, we found that lymphocyte RI was more affected by commercial 2,4-D containing 9.4% of the chemically pure 2,4-D, than with an equal concentration of the latter suggesting that other ingredients present in the commercial pesticide may be responsible or may enhance the effect of 2,4-D. Mitotic index, however, did not show any significant change with either commercial or pure 2,4-D. The lymphocytes of 12 male applicators exposed solely to 2,4-D during a 3-month period had a significantly higher RI than the same group prior to exposure and than a control group (P<0.01), in accordance with the in vitro finding of increased RI at low doses.

Cavieres MF, Jaeger J, Porter W. 2002 Nov. Developmental toxicity of a commercial herbicide mixture in mice: I. Effects on embryo implantation and litter size. Environ Health Perspect 110(11):1081-5. Abstract: We investigated the developmental toxicity in mice of a common commercial formulation of herbicide containing a mixture of 2,4-dichlorophenoxyacetic acid (2,4-D), mecoprop, dicamba, and inactive ingredients. Pregnant mice were exposed to one of four different doses of the herbicide mixture diluted in their drinking water, either during preimplantation and organogenesis or only during organogenesis. Litter size, birth weight, and crown-rump length were determined at birth, and pups were allowed to lactate and grow without additional herbicide exposure so that they could be subjected to additional immune, endocrine, and behavioral studies, the results of which will be reported in a separate article. At weaning, dams were sacrificed, and the number of implantation sites was determined. The data, although apparently influenced by season, showed an inverted or U-shaped dose-response pattern for reduced litter size, with the low end of the dose range producing the greatest decrease in the number of live pups born. The decrease in litter size was associated with a decrease in the number of implantation sites, but only at very low and low environmentally relevant doses. Fetotoxicity, as evidenced by a decrease in weight and crown-rump length of the newborn pups or embryo resorption, was not significantly different in the herbicide-treated litters.

Garaj-Vrhovac V, Zeljezic D. 2002 Jul-Aug. Assessment of genome damage in a population of Croatian workers employed in pesticide production by chromosomal aberration analysis, micronucleus assay and Comet assay. J Appl Toxicol 22(4): 249-55. Abstract: The widespread use of pesticides suggests that the evaluation of their genotoxicity should be extended using the different assays available. In the present study we used two standard cytogenetic methods (chromosomal aberration analysis and micronucleus assay) and the Comet assay as a relatively new and powerful technique. The study included 10 workers occupationally exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion) during their production and 20 control subjects with no history of exposure to any physical or chemical agents. For the chromosomal aberration analysis, whole blood was cultivated for 48 h, whereas for the micronucleus assay, whole blood was cultivated for 72 h. For the comet assay whole blood was embedded in agarose on a microscope slide, lysed with detergent, denaturated and subjected to alkaline electrophoresis. Damage to DNA was evaluated by measuring tail length and calculating the tail moment. A significantly increased number of chromatid and chromosome breaks, as well as the presence of dicentric chromosomes and chromatid exchanges in exposed subjects compared with control subjects ($P < 0.05$), was found. There was also a statistically significant difference in frequency and distribution of micronuclei between the two groups examined. In the exposed subjects the Comet assay showed a statistically significant ($P < 0.001$) increase in DNA migration. Results suggest that long-term occupational exposure to pesticides could cause genome damage in somatic cells and therefore may represent a potential hazard to human health.

Oakes DJ, Webster WS, Brown-Woodman PD, Ritchie HE. 2002 Jul. A study of the potential for a herbicide formulation containing 2,4-d and picloram to cause male-mediated developmental toxicity in rats. Toxicol

Sci 68(1):200-6.Abstract: Male Vietnam veterans have repeatedly expressed concern that exposure to herbicides in Vietnam may have caused birth defects in their offspring. The second most used herbicide was a mixture of 2,4-D and picloram called Agent White. This study is an investigation into the possible male-mediated reproductive toxicology of this herbicide. Male rats were gavaged for 5 days per week for 9 weeks with a mixture of 2,4-D and picloram called Tordon 75D(R) (the Australian derivative of Agent White). Three doses were tested; the high dose was considered the maximum tolerated dose. Each male was mated with two untreated females during weeks 2 and 3, 4 and 5, and 8 and 9 of treatment, and with four untreated females after an 11-week recovery period. Negative controls were males dosed with distilled water, and positive controls were males dosed with cyclophosphamide at 5.1 mg/kg/day. All mated females were killed on day 20 of gestation, and the fetuses were weighed and examined for either structural malformations or skeletal development. Litter size, fetal weight, and malformation rate were all unaffected by treatment. The cyclophosphamide positive controls showed the expected large increase in postimplantation loss. In general, within the limitations of the power of the study, the results did not show any evidence that exposure to a herbicide formulation containing 2,4-D and picloram is likely to cause male-mediated birth defects or other adverse reproductive outcomes.

Garabrant DH, Philbert MA. 2002 Jul. Review of 2,4-dichlorophenoxyacetic acid (2,4-D) epidemiology and toxicology. Crit Rev Toxicol 32(4):233-57.Abstract: The scientific evidence in humans and animals relevant to cancer risks, neurologic disease, reproductive risks, and immunotoxicity of 2,4-D was reviewed. Despite several thorough in vitro and in vivo animal studies, no experimental evidence exists supporting the theory that 2,4-D or any of its salts and esters damages DNA under physiologic conditions. Studies in rodents demonstrate a lack of oncogenic or carcinogenic effects following a lifetime dietary administration of 2,4-D. Epidemiologic studies provide scant evidence that exposure to 2,4-D is associated with soft tissue sarcoma, non-Hodgkin's lymphoma, Hodgkin's disease, or any other cancer. Overall, the available evidence from epidemiologic studies is not adequate to conclude that any form of cancer is causally associated with 2,4-D exposure. There is no human evidence of adverse reproductive outcomes related to 2,4-D. The available data from animal studies of acute, subchronic, and chronic exposure to 2,4-D, its salts, and esters show an unequivocal lack of systemic toxicity at doses that do not exceed renal clearance mechanisms. There is no evidence that 2,4-D in any of its forms activates or transforms the immune system in animals at any dose. At high doses, 2,4-D damages the liver and kidney and irritates mucous membranes. Although myotonia and alterations in gait and behavioral indices are observed after overwhelming doses of 2,4-D, alterations in the neurologic system of experimental animals are not observed with the administration of doses in the microgram/kg/day range. It is unlikely that 2,4-D has any neurotoxic potential at doses below those required to induce systemic toxicity.

Duffard R, Evangelista de Duffard AM. 2002 Jun. Environmental chemical compounds could induce sensitization to drugs of abuse. Ann N Y Acad Sci 965:305-13.Abstract: Chemical environment should be considered as an additional factor that influences drugs of abuse. Besides, maternal exposure to environmental chemicals has increased, and fetuses as well as neonates may be at greater risk than adults. Studies from our laboratory have described a permanent effect of the worldwide use of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) on serotonin and dopamine content in total brain and specific brain areas of adult rats born of mothers treated during lactation and fed with 2,4-D-treated diet after weaning. These animals show a modified neurotransmitter-related behavioral pattern in their developmental young and in adult age. Drugs that could be used to challenge the dopaminergic or serotoninergic systems include amphetamine or haloperidol, a postsynaptic dopamine receptor blocker. 2,4-D-exposed animals showed exacerbated response to challenges. Postnatal alterations in central dopaminergic and serotoninergic systems due to environmental chemical exposure may contribute to the enhanced/reduced behavioral sensitization to drugs of abuse.

Bortolozzi A, Duffard R, Antonelli M, Evangelista de Duffard AM. 2002 Jun. Increased sensitivity in dopamine D(2)-like brain receptors from 2,4-dichlorophenoxyacetic acid (2,4-D)-exposed and amphetamine-challenged rats. Ann N Y Acad Sci 965:314-23.Abstract: To determine whether the dopamine D(2) receptor plays a crucial role in chemically acquired sensitivity to drugs of abuse like amphetamine (AMPH) after an exposure to aryloxoalkanoic compounds, we examined in the present work the impact of AMPH (10 mg/kg, i.p.) on the dopaminergic D(2)-like receptors. Rats were exposed to 2,4-D 70 mg/kg/day from gestation day (GD) 16 to postnatal day (PND) 23. After weaning, the pups were assigned to one of the two

subgroups: T1 (fed with untreated diet until PND 90) and T2 (maintained with 2,4-D diet until PND 90). After that, an acute challenge with AMPH was administered to each animal. Rats were sacrificed at 0, 5, 24, 72, and 168 h after AMPH, and membranes of striatum (CPu), prefrontal cortex (PfC), hippocampus (H), and cerebellum (Ce) were obtained. Binding studies employing [(3)H]nemonapride showed that AMPH caused an increase in DA D(2)-like receptors of all brain areas between 5 and 24 h after the treatment, with a reduction to the basal levels one week later. The AMPH challenge to (T1 and T2) 2,4-D-exposed rats showed an alteration on receptor density depending on brain area and on sex, more than on the 2,4-D exposure time. This D(2)-like receptor density increase could explain the exacerbated behaviors of the 2,4-D-exposed and amphetamine-challenged animals, as previously observed by us. The withdrawal of 2,4-D did not produce a real reversion to basal levels of D(2)-like receptors, indicating that herbicide exposure during the preweanling period caused a sensitization and a stable DA D(2)-like receptor increase that was elicited when the system was challenged with this dopaminergic drug.

Fofana D, Kobae H, Sameshima K, Miyata K. 2002 Mar. Postnatal survival of rat offspring prenatally exposed to pure 2,4-dichlorophenoxyacetic acid (2,4-D). Congenit Anom (Kyoto) 42(1):32-5.[1]Abstract: In previous report on prenatal developmental effects of pure 2,4-dichlorophenoxyacetic acid (2,4-D) in rat, we found that this chemical was maternally toxic, embryolethal, and that it induced urogenital malformations in the fetuses. In the present report, we investigated the postnatal survival of the offspring prenatally exposed to 2,4-D during organogenesis, to determine the participation of urogenital malformations on postnatal survival. We used doses of 70 mg, 110 mg and 150 mg, which were each found to induce significant urogenital malformations, when administered in different periods of organogenesis: GD 6 to 15, GD 6 to 10, and GD 11 to 15. We found that 2,4-D has a significant influence on progeny viability by increasing the postnatal death. The kidney and urinary tract malformations induced in the fetuses might be the cause of the increased rate of postnatal death. 2,4-D did not impair the postnatal growth of the unaffected offspring.

Sreekumaran Nair R, Paulmurugan R, Singh AJ. 2002 Jan-Feb. Simple radioactive assay for the estimation of DNA breaks. J Appl Toxicol 22(1):19-23.[1]Abstract: The intactness of DNA is an important part of the normal cellular structure. Any change to the DNA in the form of breaks leads to a change in the integrity, which in turn leads to abnormality in the cellular activity. Many discrepancies have been reported among the various methods of detecting DNA damage. Here, a simple, sensitive and reproducible method has been developed for the detection of DNA breaks by radioactive labelling of 5' broken ends. The method was evaluated by studying chemically induced DNA damage by using both organochloride (2,4-dichlorophenoxyacetic acid and lindane) and organophosphorus (sevin and phosphamidon) compounds at different concentrations. Phosphamidon, one of the organophosphorus compounds studied, showed complete degradation of the DNA after treatment. Radioactive analysis of phosphamidon showed higher counts at the lowest concentration (20 microg) of the chemical when compared with the control (2752 scintillation counts per minute, scm). Studies on the chemically induced DNA breaks by radiolabelling revealed that the cumulative effect of the organophosphorus and organochloride compounds showed maximum counts in all the samples (the highest being 2904 scm) when compared with the organophosphorus and organochloride compounds studied separately (the highest being 1881 and 2260 scm, respectively). Radiolabelling studies on the blood samples of 23 pesticide workers by the newly developed assay showed a significant positive correlation (0.893) between the number of years of exposure and the scintillation counts. A maximum of 11,702 scm (for 18 years of exposure) and a minimum of 1682 scm (for 4 years of exposure) were recorded compared with 1253 scm for the negative control. This method can be used effectively for estimation of the DNA breaks, irrespective of its nature.

Zeljezic D, Garaj-Vrhovac V. 2002 Jan. Sister chromatid exchange and proliferative rate index in the longitudinal risk assessment of occupational exposure to pesticides. Chemosphere 46(2):295-303.[1]Abstract: At present, there are more than 1,000 chemicals classified as pesticides and many reports have shown that some of them have genotoxic properties. In the present longitudinal study, possible genetic damage on a population of workers occupationally exposed to a mixture of pesticides by using sister chromatid exchange (SCE) analysis has been evaluated. As an additional cytogenetic parameter, the proportion of lymphocytes that undergo one, two or three cell divisions as well as proliferative rate index have been determined. This study was performed on the exposed group of workers employed in pesticide production, simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, and malathion). The blood samples of the exposed subjects were collected in three different periods: before the

beginning of the new pesticide production period, after 8 months of everyday work in the pesticide production, and 8 months after the removal of subjects out of the production. In all three samplings, the mean value of SCE and number of cells with high sister chromatid exchange frequency (HFC) in the exposed group was significantly higher in the comparison with the control group. There were no differences in the proliferative rate index (PRI) between the control and exposed group, regardless of the sampling period. In both groups examined, the majority of lymphocytes were found in the second cell division, following cultivation. These results suggest that the increase in the number of SCE found in the exposed subjects is not the result of either cytotoxic or epigenetic action of pesticide mixture, but chronic occupational exposure to mixture of pesticides.

Sulik M, Sulik A, Barwijuk-Machala M, Pilat-Marcinkiewicz B. 2002. Fetotoxic action of 2,4-dichlorophenoxyacetic acid (2,4-D). III. Morphological changes in rat kidneys. Rocz Akad Med Bialymst 47:175-85.Abstract: The paper presents the findings of histological studies on the effect of sodium salt of 2,4-D acid on the changes within kidneys in newborn rats exposed to this herbicide in the prenatal and postnatal period. The experiment was performed on 60 Wistar rats of both sexes, up to 10 weeks of age. The animals were divided into two groups: I group (control)--18 rats fed on a standard diet and given tap water ad libitum, and group II (experimental)--42 rats, whose mothers received sodium salt of 2.4 dichlorophenoxyacetic acid in drinking water at a daily dose of c. 250 mg/kg for 2 months before fertilization and during pregnancy and lactation. The animals were killed after 24 hours, 4, 6 and 10 weeks of the experiment. The sections were taken from the kidneys, fixed in 4% formaldehyde and stained with hematoxylin and eosin. For acid and alkaline phosphatase examination, the kidney section were fixed in Backer's liquid and Gomori histoenzymatic reaction was performed. Histological examination of the first four experimental groups revealed changes in kidney tubules. Histologic changes were nonspecific and a variety of conditions. The presence vacuoles in cytoplasm and necrosis of tubular epithelial cells. Varying degrees of isometric vacuolization of proximal tubular epithelium, tubular microfocal calcification, tubular epithelial inclusion bodies and peritubular capillary congestion were observed. The observations suggest that chronic intoxication with 2,4-D acid leads to renal cell damage in kidneys more intensified in the fetal than in the postnatal period. Following herbicide withdrawal, the most pronounced changes observed in the fetus were found to regress.

Garcia G, Tagliaferro P, Bortolozzi A, Madariaga MJ, Brusco A, Evangelista de Duffard AM, Duffard R, Saavedra JP. 2001 Dec. Morphological study of 5-HT neurons and astroglial cells on brain of adult rats perinatal or chronically exposed to 2,4-dichlorophenoxyacetic acid. Neurotoxicology 22(6):733-41.Abstract: 2,4-D is a chlorophenoxyherbicide used worldwide. We have studied the morphological alterations of 5-HT neurons and glial cells in the mesencephalic nuclei of adult rats exposed to 2,4-D both perinatally (during pregnancy and lactation) and chronically (during pregnancy, lactation and after weaning) with quantitative methods. Pregnant rats were daily exposed to 70 mg/kg of 2,4-D from gestation day (GD) 16 to post-natal day (PND) 23 through diet. After weaning, pups were assigned to one of two sub-groups: T1 (fed with untreated diet until PND 90) and T2 (maintained with 2,4-D diet until PND 90). Brain sections were immunocytochemically stained using polyclonal anti-5-HT, anti-GFAP and anti-S-100 protein antibodies as cells markers. 2,4-D exposure during pregnancy and lactancy (T1 group) produced an increase in 5-HT neuronal area and immunoreactivity (IR) in the mesencephalic nuclei studied. However, with the chronical 2,4-D exposure (T2 group) only the 5-HT neuronal area from the dorsal raphe nucleus (DRN) was increased, suggesting an adaptable response of 5-HT neurons in median raphe nucleus (MRN). The presence of reactive astrocytes in mesencephalic nuclei and in hippocampus were also different for the two 2,4-D exposure designs, showing the existence of a correspondence between neuronal changes and astrogliosis. Results support evidences that 2,4-D alters the serotoninergic system and that 5-HT neurons of each mesencephalic nuclei show different responses to the 2,4-D exposure designs which are parallel to astrogliosis.

Le TN, Johansson A. 2001 Nov. Impact of chemical warfare with agent orange on women's reproductive lives in Vietnam: a pilot study. Reprod Health Matters 9(18):156-64.Abstract: During the American war in Vietnam, huge quantities of the highly toxic herbicide dioxin ('Agent Orange'), were sprayed over large areas of central and south Vietnam. In addition to polluting the environment and causing cancers and other diseases in those directly exposed to it, dioxin has caused high rates of pregnancy loss, congenital birth defects and other health problems in their children. This paper reports the findings of a pilot study in the

year 2000 among 30 Vietnamese women whose husbands and/or who themselves were exposed to Agent Orange. The aim was to develop research in order to explore the impact of chemical warfare on people's lives. Using the reproductive lifeline and semi-structured interviews, information was gathered on both partners' periods of exposure to Agent Orange, pregnancy outcomes, perceived health problems of children and experiences of living with handicapped children. The women had had a high number of miscarriages and premature births. About two-thirds of their children had congenital malformations or developed disabilities within the first years of life. Most of the families were poor, aggravated by impaired health in the men, the burden of caring for disabled children, and feelings of guilt and inferiority. The plight of 'Agent Orange families' is special and should be placed in its historical and political context.

Cooper SP, Burau K, Sweeney A, Robison T, Smith MA, Symanski E, Colt JS, Laseter J, Zahm SH. 2001 Nov. Prenatal exposure to pesticides: a feasibility study among migrant and seasonal farmworkers. Am J Ind Med 40(5):578-85.Abstract: BACKGROUND: Migrant and seasonal farmworkers have a high potential for pesticide exposures, yet are rarely included in epidemiologic studies. This study examined the feasibility of assessing prenatal exposures to pesticides and other compounds in pregnant Hispanic farmworkers. METHODS: Nine women completed a survey about work experiences during pregnancy. Maternal urine, cord blood, and placenta samples were obtained at delivery for analysis of 51 analytes, including 6 phenoxy acid or triazine herbicides, 21 organochlorine insecticides, 10 PCBs, and 14 volatile organic compounds. RESULTS: Seven of 51 analytes were found in the biological samples. DDE, DDT, dichlorbenzene, toluene, trimethylbenzene, and endosulfan sulfate were detected in cord blood samples, and 2,4-D in urine from one or more women. CONCLUSIONS: We documented the feasibility of following farmworkers to assess in utero exposure to pesticides and other contaminants, and demonstrated exposure to these compounds. Difficulties in measuring pesticides with short half lives were noted.

Madrigal-Bujaidar E, Hernandez-Ceruelos A, Chamorro G. 2001 Sep. Induction of sister chromatid exchanges by 2,4-dichlorophenoxyacetic acid in somatic and germ cells of mice exposed in vivo. Food Chem Toxicol 39(9):941-6.Abstract: 2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most widely used selective herbicides throughout the world; however, the studies that have been conducted to establish its genotoxic potential have given conflicting results. The aim of this investigation was to determine whether the herbicide increases the frequency of sister chromatid exchanges (SCEs) in bone marrow and spermatogonial cells of mice exposed in vivo. The experiment included an oral administration of 2,4-D to three groups of mice (50,100 and 200 mg/kg), as well as to a control group of animals administered with distilled water, pH 10.5 and another group injected with cyclophosphamide (50 mg/kg). In somatic cells, the results showed a significant SCE increase with the two high doses tested, a response that was manifested in a dose-dependent manner. With regard to the mitotic index and the cell proliferation kinetics, there were no modifications exerted by 2,4-D; however, cyclophosphamide induced cytotoxic damage and a cell-cycle delay. With respect to the germ cells, the genotoxic results were similar to those described earlier; that is, there was a significant SCE increase induced by the two high 2,4-D doses tested and a higher genotoxic damage was observed in the animals treated with cyclophosphamide. Our investigation established that 2,4-D is a moderate genotoxicant in mice treated in vivo with high doses, and suggests a minor hazard for humans in the present conditions of its use.

Lee K, Johnson VL, Blakley BR. 2001 Aug 13. The effect of exposure to a commercial 2,4-D formulation during gestation on the immune response in CD-1 mice. Toxicology 165(1):39-49.Abstract: Pregnant CD-1 mice were administered a commercial 2,4-dichlorophenoxyacetic acid (2,4-D) formulation on days 6-16 days of gestation, in drinking water at concentrations ranging from 0 to 1.0% of the formulated product, equivalent to approximately 0-650 mg/kg per day expressed as the amine derivative. The effect of 2,4-D on immune function was evaluated in offspring 7 weeks after birth. The dams tolerated repeated 2,4-D exposure in drinking water without difficulty. The offspring exhibited decreased body weight with minor reductions in the kidney weights in the 0.1 and 1.0% 2,4-D treatment groups. A generalized suppression of lymphocyte stimulation by concanavalin A (Con A) was observed at high dose of commercial 2,4-D formulation (1.0%). Cytometric studies of the lymphocyte subpopulations demonstrated an increased relative count of B cells and reduced T cytotoxic or suppressor cells in the 1.0% formulation. The humoral immune response, antibody production against sheep red blood cells and peritoneal macrophage phagocytic function, were not altered by 2,4-D. Since the immune alterations in the offspring were observed many weeks after exposure, it appears as though 2,4-D exposure during gestation causes permanent changes in cell types associated

with immune function. Since 2,4-D is not considered a persistent chemical, it is unlikely that 2,4-D residues are contributing significantly to the observed immune alterations. The immune alterations were observed only in the higher treatment groups. Therefore, the impact on human and animal health from an immune perspective, which would be encountered following normal application in the environment, would be minimal.

Arbuckle TE, Lin ZQ, Mery LS. 2001 Aug. An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an ontario farm population. Environ Health Perspect 109(8):851-857. Abstract: The toxicity of pesticides on human reproduction is largely unknown-particularly how mixtures of pesticide products might affect fetal toxicity. The Ontario Farm Family Health Study collected data by questionnaire on the identity and timing of pesticide use on the farm, lifestyle factors, and a complete reproductive history from the farm operator and eligible couples living on the farm. A total of 2,110 women provided information on 3,936 pregnancies, including 395 spontaneous abortions. To explore critical windows of exposure and target sites for toxicity, we examined exposures separately for preconception (3 months before and up to month of conception) and postconception (first trimester) windows and for early (< 12 weeks) and late (12-19 weeks) spontaneous abortions. We observed moderate increases in risk of early abortions for preconception exposures to phenoxy acetic acid herbicides [odds ratio (OR) = 1.5; 95% confidence interval (CI), 1.1-2.1], triazines (OR = 1.4; 95% CI, 1.0-2.0), and any herbicide (OR = 1.4; 95% CI, 1.1-1.9). For late abortions, preconception exposure to glyphosate (OR = 1.7; 95% CI 1.0-2.9), thiocarbamates (OR = 1.8; 95% CI, 1.1-3.0), and the miscellaneous class of pesticides (OR = 1.5; 95% CI 1.0-2.4) was associated with elevated risks. Postconception exposures were generally associated with late spontaneous abortions. Older maternal age (> 34 years of age) was the strongest risk factor for spontaneous abortions, and we observed several interactions between pesticides in the older age group using Classification and Regression Tree analysis. This study shows that timing of exposure and restricting analyses to more homogeneous endpoints are important in characterizing the reproductive toxicity of pesticides.

Amer SM, Aly FA. 2001 Jul 25. Genotoxic effect of 2,4-dichlorophenoxy acetic acid and its metabolite 2,4-dichlorophenol in mouse. Mutat Res 494(1-2):1-12. Abstract: The cytogenetic effect of 2,4-dichlorophenoxy acetic acid (2,4-D) and its metabolite 2,4-dichlorophenol (2,4-DCP) was studied in bone-marrow, germ cells and sperm head abnormalities in the treated mice. Swiss mice were treated orally by gavage with 2,4-D at 1.7, 3.3 and 33 mg kg(-1)BW (1/200, 1/100 and 1/10 of LD(50)). 2,4-DCP was intraperitoneally (i.p.) injected at 36, 72 and 180 mg kg(-1)BW (1/10, 1/5, 1/2 of LD(50)). A significant increase in the percentage of chromosome aberrations in bone-marrow and spermatocyte cells was observed after oral administration of 2,4-D at 3.3 mg kg(-1)BW for three and five consecutive days. This percentage increased and reached 10.8+/-0.87 ($P<0.01$) in bone-marrow and 9.8+/-0.45 ($P<0.01$) in spermatocyte cells after oral administration of 2,4-D at 33 mg kg(-1)BW for 24 h. This percentage was, however, lower than that induced in bone-marrow and spermatocyte cells by mitomycin C (positive control). 2,4-D induced a dose-dependent increase in the percentage of sperm head abnormalities. The genotoxic effect of 2,4-DCP is weaker than that of 2,4-D, as indicated by the lower percentage of the induced chromosome aberrations (in bone-marrow and spermatocyte cells) and sperm head abnormalities. Only the highest tested concentration of 2,4-DCP (180 mg kg(-1)BW, 1/2 LD(50)) induced a significant percentage of chromosome aberrations and sperm head abnormalities after i.p. injection. The obtained results indicate that 2,4-D is genotoxic in mice in vivo under the conditions tested. Hence, more care should be given to the application of 2,4-D on edible crops since repeated uses may underlie a health hazard.

Ozaki K, Mahler JF, Haseman JK, Moomaw CR, Nicolette ML, Nyska A. 2001 Jul-Aug. Unique renal tubule changes induced in rats and mice by the peroxisome proliferators 2,4-dichlorophenoxyacetic acid (2,4-D) and WY-14643. Toxicol Pathol 29(4):440-50. Abstract: Peroxisome proliferators are non-mutagenic carcinogens in the liver of rodents, acting both as initiators and promoters. The National Toxicology Program (NTP) conducted a study of several peroxisome proliferators (PPs), including Wyeth (WY)-14643 as a prototypical PP and 2,4-dichlorophenoxyacetic acid (2,4-D) as a weak PP, in Sprague-Dawley rats. B6C3F1 mice, and Syrian hamsters. In the kidney, an unusual change was observed in the outer stripe of the outer medulla, especially in rats treated with 2,4-D or WY-14643. This change was characterized by foci of tubules that were partially or completely lined by basophilic epithelial cells with decreased cytoplasm and high nuclear density. Changes typical of chronic nephropathy such as interstitial fibrosis or

basement membrane thickening were not associated with these foci. Results of immunohistochemical staining for catalase and cytochrome P-450 4A in the kidney indicated increased staining intensity in renal tubular epithelial cells primarily in the region where the affected tubules were observed: however, the altered cells were negative for both immunohistochemical markers. Ultrastructurally, affected cells had long brush borders typical of the P3 tubule segment. The most distinguishing ultrastructural change was a decreased amount of electronlucent cytoplasm that contained few differentiated organelles and, in particular, a prominent reduced volume and number of mitochondria; changes in peroxisomes were not apparent. In addition to the lesion in rats, mice treated with the highest dose of 2,4-D, but not WY-14643, manifested similar renal tubular changes as seen by light microscopy. Neither chemical induced renal tubular lesions in hamsters. Hepatocellular changes characteristic of PPs were present in all 3 species treated with WY-14643, but not 2,4-D. These results indicate that the rat is the species most sensitive to the nephrotoxic effects of PPs and there is a site specificity to this toxicity related to areas of PP-related enzyme induction. Although 2,4-D is considered a weak PP for the liver, it was the most effective at inducing renal lesions, indicating that the toxic potency of various PPs will depend on the target organ.

Garry VF, Tarone RE, Kirsch IR, Abdallah JM, Lombardi DP, Long LK, Burroughs BL, Barr DB, Kesner JS. 2001 May. Biomarker correlations of urinary 2,4-D levels in foresters: genomic instability and endocrine disruption. Environ Health Perspect 109(5):495-500.Abstract: Forest pesticide applicators constitute a unique pesticide use group. Aerial, mechanical-ground, and focal weed control by application of herbicides, in particular chlorophenoxy herbicides, yield diverse exposure scenarios. In the present work, we analyzed aberrations in G-banded chromosomes, reproductive hormone levels, and polymerase chain reaction-based V(D)J rearrangement frequencies in applicators whose exposures were mostly limited to chlorophenoxy herbicides. Data from appliers where chlorophenoxy use was less frequent were also examined. The biomarker outcome data were compared to urinary levels of 2,4-dichlorophenoxyacetic acid (2,4-D) obtained at the time of maximum 2,4-D use. Further comparisons of outcome data were made to the total volume of herbicides applied during the entire pesticide-use season.Twenty-four applicators and 15 minimally exposed foresters (control) subjects were studied. Categorized by applicator method, men who used a hand-held, backpack sprayer in their applications showed the highest average level (453.6 ppb) of 2,4-D in urine. Serum luteinizing hormone (LH) values were correlated with urinary 2,4-D levels, but follicle-stimulating hormone and free and total testosterone were not. At the height of the application season; 6/7 backpack sprayers, 3/4 applicators who used multinozzle mechanical (boom) sprayers, 4/8 aerial applicators, and 2/5 skidder-radiarc (closed cab) appliers had two or more V(D)J region rearrangements per microgram of DNA. Only 5 of 15 minimally exposed (control) foresters had two or more rearrangements, and 3 of these 5 subjects demonstrated detectable levels of 2,4-D in the urine. Only 8/24 DNA samples obtained from the exposed group 10 months or more after their last chlorophenoxy use had two rearrangements per microgram of DNA, suggesting that the exposure-related effects observed were reversible and temporary. Although urinary 2,4-D levels were not correlated with chromosome aberration frequency, chromosome aberration frequencies were correlated with the total volume of herbicides applied, including products other than 2,4-D. In summary, herbicide applicators with high urinary levels of 2,4-D (backpack and boom spray applications) exhibited elevated LH levels. They also exhibited altered genomic stability as measured by V(D)J rearrangement frequency, which appears reversible months after peak exposure. Though highly detailed, the limited sample size warrants cautious interpretation of the data.

Charles JM, Hanley TR Jr, Wilson RD, van Ravenzwaay B, Bus JS. 2001 Mar. Developmental toxicity studies in rats and rabbits on 2,4-dichlorophenoxyacetic acid and its forms. Toxicol Sci 60(1):121-31.Abstract: The potential for 2,4-D and its salts and esters to induce developmental toxicity was investigated in rats (8 studies) and rabbits (7 studies). Maternal toxicity associated with exposure was dependent on the dose level expressed as 2,4-D acid equivalents. The severity of the maternal effect was correlated to the 2,4-D acid-equivalent dose, with increasing dose levels that exceeded renal clearance causing increasingly more severe maternal effects. In both species, maternal body weight effects began to be manifested at dose levels of 30 mg 2,4-D acid equivalent/kg/day. At higher dose levels (50-75 mg/kg/day in rats and 75-90 mg/kg/day in rabbits), body weights and feed consumption were more severely affected. At dose levels > or =90 mg/kg/day in rats, clinical signs of toxicity (ataxia, muscular stiffness, and decreased motor activity) and mortality were noted. The no-observed-adverse-effect level (NOAEL) for maternal toxicity in both species across the family of 2,4-D salts and esters was approximately 10 mg/kg/day. Significantly decreased fetal body weights and increased fetal variations were seen in rats only at maternally toxic dose levels in excess

of 90 mg/kg/day acid equivalent. At maternally toxic doses in rabbits, embryonal and fetal development were essentially unaffected. There were no effect on maternal reproductive measures such as litter size, resorption rates, or fetal body weights, and there was no evidence of teratogenic activity. **In summary, equivalent toxicity of the salts and esters is consistent with rapid and complete metabolic conversion to 2,4-D acid**. No adverse fetal effects were noted at dose levels that did not also produce evidence of maternal toxicity or exceed renal clearance of 2,4-D indicating that the developing rat and rabbit fetus were not uniquely sensitive to 2,4-D and its forms.

Alpoz AR, Tosun N, Eronat C, Delen N, Sen BH . 2001 Jan. Effects of 2,4-dichlorophenoxy acetic acid dimethyl amine salt on dental hard tissue formation in rats. Environ Int 26(3):137-42. Abstract: 2,4-Dichlorophenoxy acetic acid dimethyl amine salt (2,4-D DMA), as one of the phenoxy acids, is used as a herbicide mainly against broad-leaf weeds in cereal crops, sugar cane, and on turf, pasture, and non-crop land. Some formulations of 2,4-D may be contaminated with dioxins. Recently, it has been shown that chlorinated organic compounds, dioxins, and furans are present in mother's milk and may cause developmental defects in children's teeth. Therefore, we aimed to evaluate the effects of 2,4-D DMA on odontogenesis in rats. 2,4-D DMA was given orally combined with rat food to pregnant albino rats. Each group consisted of two pregnant rats and, 0 (control, group A), 25 ppm (group B), 50 ppm (group C), and 100 ppm (group D) 2,4-D DMA was given to each pregnant rat as daily intake. 2,4-D DMA affected young rat's dental development and dose-related findings were found in experimental groups. The odontoblast layer was irregular and globular dentin formation was present in Groups B, C, and D but not in the control group. Thickness of enamel decreased in Groups C and D. The results of the study have shown that 2,4-D DMA could disturb dental development in rats even in relatively low doses. It is concluded that environmental contaminants such as chlorinated organic pesticides may play an important role in infant's dental development when taken via mother's milk.

Gunier RB, Harnly ME, Reynolds P, Hertz A, von Behren J. 2001. Agricultural pesticide use in California: Pesticide prioritization, use densites, and population distribution for a childhood cancer study. Environ Health Perspect 109(10):1071-1078. Abstract: Several studies have suggested an association between childhood cancer and pesticide exposure. California leads the nation in agricultural pesticide use. A mandatory reporting system for all agricultural pesticide use in the state provides information on the active ingredient, amount used, and location. We calculated pesticide use density to quantify agricultural pesticide use in California block groups for a childhood cancer study. Pesticides with similar toxicologic properties (probable carcinogens, possible carcinogens, genotoxic compounds, and developmental or reproductive toxicants) were grouped together for this analysis. To prioritize pesticides, we weighted pesticide use by the carcinogenic and exposure potential of each compound. The top-ranking individual pesticides were propargite, methyl bromide, and trifluralin. We used a geographic information system to calculate pesticide use density in pounds per square mile of total land area for all United States census-block groups in the state. Most block groups (77%) averaged less than 1 pound per square mile of use for 1991-1994 for pesticides classified as probable human carcinogens. However, at the high end of use density (> 90th percentile), there were 493 block groups with more than 569 pounds per square mile. Approximately 170,000 children under 15 years of age were living in these block groups in 1990. The distribution of agricultural pesticide use and number of potentially exposed children suggests that pesticide use density would be of value for a study of childhood cancer.

Charles JM, Cifone MA, Lawlor T, Murli H, Young RR, Leeming NM. 2000 Dec 20. Evaluation of the in vitro genetic toxicity of 4-(2, 4-dichlorophenoxy)butyric acid. Mutat Res 472(1-2):75-83. Abstract: The herbicide 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB) is principally used in the USA on peanuts, soybeans and alfalfa. In Europe, it is used on undersown spring barley and grassland (with clover). The genetic toxicity in vitro of the dimethylamine salt of 2,4-DB was examined by employing a range of end points including gene mutation in bacteria (Ames test) and mammalian cell cultures (CHO/HGPRT assay), cytogenetic abnormalities in mammalian cells (CHO/chromosomal aberration assay), and induction of DNA damage and repair in rat hepatocytes. There were no indications of genotoxic potential for 2,4-DB in the first three of these assays. One of the two criteria for a positive response in the UDS assay was exceeded but the increases did not exceed the second criteria for a positive response. The test material was therefore evaluated as weakly active in this assay. The weight of the evidence clearly indicates that 2, 4-DB is not genotoxic to mammals and are consistent with the reported lack of carcinogenic potential for 2,4-DB

in both mice and rats.

Venkov P, Topashka-Ancheva M, Georgieva M, Alexieva V, Karanov E. 2000 Nov. Genotoxic effect of substituted phenoxyacetic acids. Arch Toxicol 74(9):560-6.Abstract: The potential toxic and mutagenic action of 2,4-dichlorophenoxyacetic acid has been studied in different test systems, and the obtained results range from increased chromosomal damage to no effect at all. We reexamined the effect of this herbicide by simultaneous using three tests based on yeast, transformed hematopoietic, and mouse bone marrow cells. The results obtained demonstrated that 2,4-dichlorophenoxyacetic acid has cytotoxic and mutagenic effects. The positive response of yeast and transformed hematopoietic cells was verified in kinetics and dose-response experiments. The analysis of metaphase chromosomes indicated a statistically proved induction of breaks, deletions, and exchanges after the intraperitoneal administration of 2,4-dichlorophenoxyacetic acid in mice. The study of phenoxyacetic acid and its differently chlorinated derivatives showed that cytotoxicity and mutagenicity are induced by chlorine atoms at position 2 and/or 4 in the benzene ring. The mutagenic effect was abolished by introduction of a third chlorine atom at position 5. Thus 2,4,5-trichlorophenoxyacetic acid was found to have very weak, if any mutagenic effect; however, the herbicide preserved its toxic effect.

Lin N, Garry VF. 2000 Jul 28. In vitro studies of cellular and molecular developmental toxicity of adjuvants, herbicides, and fungicides commonly used in Red River Valley, Minnesota. J Toxicol Environ Health A 60 (6):423-39.Abstract: Recent epidemiologic studies showed increased frequency of birth defects in pesticide applicators and general population of the Red River Valley, Minnesota. These studies further indicated that this crop growing area used more chlorophenoxy herbicides and fungicides than elsewhere in Minnesota. Based on frequency of use and known biology, certain herbicides, pesticide additives, fungicides, and mycotoxins are suspect agents. To define whether these agents affect developmental endpoints in vitro, 16 selected agrochemicals were examined using the MCF-7 breast cancer cell line. In the flow cytometric assay, cell proliferation in this estrogen-responsive cell line indicates xenobiotic-mediated estrogenic effects. Cell viability, morphology, ploidy, and apoptosis were incorporated in this assay. Data showed that the adjuvants X-77 and Activate Plus induced significant cell proliferation at 0.1 and 1 microg/ml. The commercial-grade herbicides 2,4-D LV4 and 2,4-D amine induced cell proliferation at 1 and 10 microg/ml. The reagent-grade 2,4-D products failed to induce proliferation over the same concentration range, suggesting that other ingredients in the commercial products, presumably adjuvants, could be a factor in these results. The fungicides triphenyltin and mancozeb induced apoptosis at concentrations of 4.1 microg/ml (10(-5) M) and 50 microg/ml, respectively. Triphenyltin also induced aneuploidy (C2/M arrest) at 0.41 microg/ml (10(-6) M). Data provide a mechanistic step to understanding human reproductive and developmental effects in populations exposed to these agrochemicals, and initiative to focusing limited resources for future in vivo animal developmental toxicity studies.

Lee K, Johnson VJ, Blakley BR. 2000 Jun. The effect of exposure to a commercial 2,4-D herbicide formulation during gestation on urethan-induced lung adenoma formation in CD-1 mice. Vet Hum Toxicol 42(3):129-32.Abstract: Female CD-1 mice were exposed to a commercial amine formulation of 2,4-dichlorophenoxyacetic acid (2,4-D) on days 6-16 of gestation in drinking water at concentrations ranging from 0 to 1.0% of the formulated product, equivalent to approximately 0-650 mg/kg/d expressed as the amine derivative. The effect of 2,4-D on urethan-induced pulmonary adenoma formation was evaluated in female offspring 19 w after birth. Urethan-induced sleeping times observed following ip injection of 1.5 mg urethan/g bw 7 w after birth were not altered by 2,4-D ($p = 0.10$), indicating that 2,4-D did not affect the rate of urethan elimination. 2,4-D exposure did not affect the number of tumors produced ($p = 0.58$), but did reduce the mean tumor diameter in the highest dose group ($p < 0.01$). This minor antineoplastic activity of 2,4-D may be related, in part, to inhibitory effects of 2,4-D on various enzymatic or metabolic pathways, essential for cellular growth and tissue development. Since exposure to 2,4-D during pregnancy had little impact of tumor production, it is unlikely that persistent alteration to developing immune cells involved in the cell-mediated immunosurveillance mechanisms occurred. The subtle alteration in tumor size and the mild impairment of growth in the offspring were observed almost exclusively in the highest treatment group. Since this level of exposure is well in excess of those associated with normal application of 2,4-D, the hazard to non-target mammalian populations appears minimal.

Hogan DA, Smith SR, Saari EA, McCracken J, Hausinger RP. 2000 Apr 28. Site-directed mutagenesis of 2,4-

dichlorophenoxyacetic acid/alpha-ketoglutarate dioxygenase - identification of residues involved in metallocenter formation and substrate binding. J Biol Chem 275(17):12400-12409.[1]Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D)/alpha-ketoglutarate (alpha-KG) dioxygenase (TfdA) is an Fe(II)-dependent enzyme that catalyzes the first step in degradation of the herbicide 2,4-D, The active site structures of a small number of enzymes within the alpha-KG-dependent dioxygenase superfamily have been characterized and shown to have a similar HXDX50-70HX10RXS arrangement of residues that make up the binding sites for Fe(II) and alpha-KG. TfdA does not have obvious homology to the dioxygenases containing the above motif but is related in sequence to eight other enzymes in the superfamily that form a distinct consensus sequence (HX(D/E)X138-207 HX10R/K). Variants of TfdA were created to examine the roles of putative metal-binding residues and the functions of the other seven histidines in this protein. The H167A, H200A, H213A, H245A, and H262A forms of TfdA formed inclusion bodies when overproduced in Escherichia coli DH5 alpha; however, these proteins were soluble when fused to the maltose-binding protein (MBP). MBP-TfdA exhibited kinetic parameters similar to the native enzyme. The H8A and H235A variants were catalytically similar to wild-type TfdA. MBP-H213A and H216A TfdA have elevated K-m values for 2,4-D, and the former showed a decreased k(cat), suggesting these residues may affect substrate binding or catalysis, The H113A, D115A, MBP-H167A, MBP-H200A, MBP-H245A and MBP-H262A variants of TfdA were inactive. Gel filtration analysis revealed that the latter two proteins were highly aggregated. The remaining four inactive variants were examined in their Cu(II)-substituted forms by EPR and electron spin-echo envelope modulation (ESEEM) spectroscopic methods. Changes in EPR spectra upon addition of substrates indicated that copper was present at the active site in the H113A and D115A variants. ESEEM analysis revealed that two histidines are bound equatorially to the copper in the D115A and MBP-H167A TfdA variants. The experimental data and sequence analysis lead us to conclude that His-113, Asp-115, and His-262 are likely metal ligands in TfdA and that His-213 may aid in catalysis or binding of 2,4-D.

Sturtz N, Evangelista de Duffard AM, Duffard R. 2000 Feb-Apr. Detection of 2,4-dichlorophenoxyacetic acid (2,4-D) residues in neonates breast-fed by 2,4-D exposed dams. Neurotoxicology 21(1-2):147-54.[1]Abstract: Knowing early nutritional status have been shown to be an important factor in determining the activity level of rats later in life, we studied offspring of dams which had received 50, 70 or 700 mg/kg of 2,4-Dichlorophenoxyacetic acid (2,4-D) during nursing. Neonatal tissues and the stomach content (milk) were examined up to 16 post natal days to detect body and organs weight alterations and 2,4-D residues after 2,4-D maternal dosing every-other-day, from post natal day 1. We detected 2,4-D residues in stomach content, blood, brain and kidney of 4-day-old neonates breast-fed by 2,4-D exposed mothers and onward. 2,4-D residues were dose- and exposure-time-dependent. The highest dose impaired body growth, as well as low tissue weights and diminished stomach contents. Levels of 2,4-D residues in stomach content, blood, kidney and brain of post natal rats (age PD 4-PD 16) fed through lactation from dams treated with 2,4-D demonstrated that 2,4-D was transferred to the neonates and the diminished body and tissues weight during this developmental period could be due to a diminished milk intake or/and to the direct 2,4-D toxic effect. Besides, when the herbicide treatment (100 mg 2,4-D/kg) was withdrawn from the dams, 2,4-D residues remained in the stomach content of neonates for at least one week. This fact indicated that dams continued depurating the herbicide through their milk.

Mikov I, Milosevic M, Mikov A, Mikov M. 2000. Increased urinary excretion of thioethers as a marker for detecting exposure to herbicide containing 2,4-dichlorophenoxyacetic acid dimethylamine - experimental study on mice. Annals of Agricultural & Environmental Medicine 7(1):61-63.[1]Abstract: The possibility that urinary thioethers concentration might be a marker for detecting exposure to herbicide containing 2,4-dichlorophenoxyacetic acid dimethylamine (2,4-DMA) was investigated in animals. Mice were treated with the herbicide containing 2,4-DMA consecutively for 4 days. Urinary concentrations of thioethers related either to body weight or creatinine concentration in urine in the group of animals treated with herbicide were significantly higher compared to control group. Results suggest that thioethers determination in urine might be a noninvasive and simple method for detecting exposure to herbicide containing 2,4-DMA.

Gollapudi BB, Charles JM, Linscombe VA, Day SJ, Bus JS. 1999 Jul 21. Evaluation of the genotoxicity of 2,4-dichlorophenoxyacetic acid and its derivatives in mammalian cell cultures. Mutat Res 444(1):217-25.[1]Abstract: 2,4-dichlorophenoxyacetic acid and its derivatives (collectively known as 2,4-D) are herbicides used to control a wide variety of broadleaf and woody plants. The genetic toxicity of an ester

(2,4-D 2-butoxyethylester) and two salts (2,4-D isopropylamine and 2,4-D triisopropanolamine) was investigated in cultured mammalian cells. The end points used were the induction of chromosomal aberrations in primary cultures of rat lymphocytes and forward mutations at the HGPRT locus of Chinese hamster ovary cells. There was no evidence of genotoxicity for the test materials in the experimental systems used. These results were consistent with the general lack of genotoxic potential for 2,4-D in a number of other test systems.

Charles JM, Cunny HC, Wilson RD, Ivett JL, Murli H, Bus JS, Gollapudi B. 1999 Jul 21. In vivo micronucleus assays on 2,4-dichlorophenoxyacetic acid and its derivatives. Mutat Res 444(1):227-34. Abstract: The potential for 2,4-D and seven of its salts and esters to induce cytogenetic abnormalities in mammalian cells in vivo was investigated in the mouse bone marrow micronucleus test. All the test materials were administered to male and female mice by oral gavage and the frequencies of micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow were determined at intervals of 24, 48 and 72 h following dosing. There were no significant increases in the incidence of MN-PCE in the treated mice at any of the bone marrow sampling times. These results are consistent with the reported lack of in vitro genetic toxicity for these materials in various in vitro genotoxicity assays as well as the absence of carcinogenic potential for 2,4-D in both mice and rats.

Charles JM, Cunny HC, Wilson RD, Bus JS, Lawlor TE, Cifone MA, Fellows M, Gollapudi B. 1999 Jul 21. Ames assays and unscheduled DNA synthesis assays on 2, 4-dichlorophenoxyacetic acid and its derivatives. Mutat Res 444(1):207-16. Abstract: 2,4-dichlorophenoxyacetic acid and several of its derivatives (collectively known as 2,4-D) are herbicides used to control a wide variety of broadleaf and woody plants. The genetic toxicity in vitro of 2,4-D and seven of its salts and esters were examined by employing gene mutation in bacteria (Ames test) and induction of DNA damage and repair in rat hepatocytes. In addition, an in vivo unscheduled DNA synthesis (UDS) assay was performed on 2,4-D. There were no indications of genotoxic potential for 2,4-D acid, or any of its derivatives, in these assays. These results are consistent with the reported lack of carcinogenic potential for 2,4-D in both mice and rats.

Bortolozzi AA, Duffard RO, Evangelista de Duffard AM. 1999 Jul-Aug. Behavioral alterations induced in rats by a pre- and postnatal exposure to 2,4-dichlorophenoxyacetic acid. Neurotoxicol Teratol 21(4):451-65. Abstract: The purpose of this study was to determine whether the behavioral development pattern was altered by a pre- and postnatal exposure to 2,4-Dichlorophenoxyacetic acid (2,4-D). Pregnant rats were daily orally exposed to 70 mg/kg/day of 2,4-D from gestation day (GD) 16 to postnatal day (PND) 23. After weaning, the pups were assigned to one of the two subgroups: T1 (fed with untreated diet until PND 90) and T2 (maintained with 2,4-D diet until PND 90). Effects on offsprings were evaluated with a neurotoxicological test battery. Neuromotor reflexes, spontaneous motor activity, serotonin syndrome, circling, and catalepsy were analyzed during various postnatal ages. 2,4-D neonatal exposure induced delay of the ontogeny of righting reflex and negative geotaxis accompanied by motor abnormalities, stereotypic behaviors (excessive grooming and vertical head movements), and hyperactivity in the open field. Adult rats of both sexes (T2 group) showed a diminution of ambulation and rearing, while excessive grooming responses were only observed in T2 males. Besides, these animals manifested serotonin syndrome behaviors, catalepsy, and right-turning preference. Some behaviors were reversible, but others were permanent, and some were only expressed after pharmacological challenges.

Garry VF, Burroughs B, Tarone R, Kesner JS. 1999 Jan-Mar. Herbicides and adjuvants: an evolving view. Toxicol Ind Health 15(1-2):159-67. Abstract: The present report examines the in vitro genotoxicity (micronucleus assay) of herbicides and adjuvants and reports on an in vivo human study on potential endocrine effects of pesticides, including herbicides. Adjuvants are used in conjunction with 2,4-dichlorophenoxy acetic acid (2,4-D) and other herbicides. Earlier pesticide applier survey results (n = 709) show that 59% of the applicators used adjuvants, and the majority of this group used paraffinic oils and/or surfactant mixtures. As a beginning effort to explore the role of adjuvants and herbicides in hormonally based reproductive effects, a prospective, controlled study was performed to analyze blood specimens from three different exposure groups (applicators using herbicides only; applicators using both herbicides and insecticides; and applicators using fumigants in addition to herbicides and insecticides; and a control group composed of other agricultural workers including organic farmers). The applicators and controls were age- and smoking-matched. Study subjects (n = 78) were tested before, during, and after completion of pesticide application

season for the effects of pesticide products on hormone levels in the bloodstream. Of the applicator exposure groups examined, only the herbicide group showed significant endocrinologic differences from controls. Free testosterone levels were significantly elevated in post-season measurements ($p = 0.032$), and follicle-stimulating hormone (FSH) was significantly decreased at the height of the season ($p = 0.016$) and in the post-season ($p = 0.010$) as compared to controls. These endocrinologic findings are discussed in terms of their possible relationship to potential endocrine effects of herbicides, herbicide contaminants, and adjuvants. In vitro genotoxicity examination compared four different commercially available surfactant mixtures with 12 different commercial herbicide products, including six different chlorophenoxy herbicides. Only one herbicide yielded a significant dose-response curve. All four adjuvants showed positive dose-response effects. These preliminary data suggest that adjuvants are not inert but are toxicologically active components added to herbicide mixtures. Whether adjuvant toxicant effects are additive or are independent of herbicide effects is poorly understood.

Crain DA, Spiteri ID, Guillette LJ Jr. 1999 Jan-Mar. The functional and structural observations of the neonatal reproductive system of alligators exposed in ovo to atrazine, 2,4-D, or estradiol. Toxicol Ind Health 15(1-2):180-5.Abstract: Wild alligators exposed to persistent organochlorine contaminants, municipal waste compounds, and contemporary-use herbicides exhibit reproductive alterations that are thought to be caused by endocrine disruption. This study tests the hypothesis that these alterations, at least in part, result from exposure of alligator embryos to contemporary-use herbicides. Alligator eggs were collected early in development, exposed to estradiol-17 beta, atrazine, or 2,4-D (at dosages of 0.14, 1.4, and 14 ppm, plus a dosage of 0.014 ppm for estradiol-17 beta only) before the period of gonadal differentiation, and incubated at a temperature that would produce either 100% males or 100% females. Analysis of histology was performed on the gonads and reproductive tracts of hatchlings. In females, epithelial cell height of the Mullerian duct and medullary regression of the ovary were assessed, whereas in males, sex-cord diameter was measured. Eggs incubated at the female-determining temperature produced all female hatchlings, whereas the estradiol-17 beta treatments caused the production of females at the male-determining temperature. Neither atrazine nor 2,4-D had this effect. Both Mullerian duct epithelial cell height and medullary regression were increased in estradiol-treated animals, but no differences were noted between herbicide-treated alligators and controls. A previous study found that male alligators exposed to 14 ppm atrazine had elevated gonadal aromatase activity, but there was no difference in sex-cord diameter in this or any other treatment group. Additionally, we observed that hepatic aromatase activity was not altered by in ovo exposure to any of the treatments. These results indicate that these herbicides alone are not responsible for the gonadal abnormalities previously reported for juvenile alligators from Lake Apopka and emphasize the importance of analyzing both the function (i.e., steroidogenic enzyme activity) and the structure (i.e., histological analysis) of the reproductive system. Structural assessment alone may be insufficient for detecting subtle endocrine alterations.

Cox C. 1999. Herbicide factsheet: 2,4-D: Toxicology, part 2. Journal of Pesticide Reform 19(2):14-19.Abstract: 2,4-D is a widely used herbicide in the phenoxy family. Exposure has been linked with low-qualtiy sperm, birth defects, and non-Hodgkin's lymphoma. It disrupts the normal functions of hormone systems.

Kaioumova DF, Khabutdinova LKh. 1998 Oct-Nov. Cytogenetic characteristics of herbicide production workers in Ufa. Chemosphere 37(9-12):1755-9.Abstract: In the present study, we investigated the effect of dioxin-containing products on the cytogenetic characteristics of peripheral blood lymphocytes of herbicide plant workers in Ufa. We found that the mean incidence of cells with chromosomal abberations (CHA) was two fold higher in the herbicide plant workers than the mean incidence level of controls groups consisting of people with no professional contact to herbicides or hospital stuff working in the close vicinity of the herbicide plant in Ufa (for both cases: $p < 0.05$). Moreover, the mean CHA cell incidence in the controls groups was also two times higher than the average level of spontaneous abberations in humans. The chemical herbicides 2,4,5-trichlorphenol (2,4,5-T) and 2,4-dichlorophenoxiacetic acid (2,4-D) appeared to affect various cellular cycle phases. Chromosomal type abberations occurred in the G0 stage of cellular cycle and chromatic type aberrations in the G2 stage. In the S stage, the aberrations of both types were observed. Our results indicate that the herbicides 2,4,5-T and 2,4-D have mutagenic effects in humans.

Michalek JE, Rahe AJ, Boyle CA. 1998 Mar. Paternal dioxin, preterm birth, intrauterine growth retardation, and infant death . Epidemiology 9(2):161-7.Abstract: We studied paternal exposure to Agent Orange and its

dioxin contaminant (2,3,7,8-tetrachlorodibenzo-p-dioxin) and preterm birth, intrauterine growth retardation, or infant death in veterans of Operation Ranch Hand, the unit responsible for spraying herbicides during the Vietnam war. A Comparison group of Air Force veterans who served in Southeast Asia during the same time period and who were not occupationally exposed to herbicides was included. We studied children conceived during or after the father's service in Southeast Asia and based exposure on paternal dioxin measured in 1987 or 1992 extrapolated to the time of conception of the child. We assigned each child to one of four exposure categories: Comparison and three Ranch Hand categories (Background, Low, High). Children in the High (relative risk = 1.3) and Background (relative risk = 1.4) categories were at increased risk of preterm birth. The risk of intrauterine growth retardation was not increased in any exposure category. The risk of infant death was increased in all Ranch Hand children, with the greatest increases in the High (relative risk = 4.5) and Background (relative risk = 3.2) categories. These patterns indicate that the increases in the relative risk of preterm birth and infant death may not be related to paternal dioxin level.

Sulik M, Pilat-Marcinkiewicz B, Sulik A, Barwijuk-Machala M, Sulkowska M, Baltaziak M, Klepacka J. 1998. Fetotoxic effect of 2,4-dichlorophenoxyacetic acid (2,4-D) in rats. Rocz Akad Med Bialymst 43:298-308. Abstract: The fetotoxic effect of 2,4-dichlorophenoxyacetic acid (2,4-D) was investigated. Histological and histochemical changes in the liver of newborn, jung and adult rats exposed to the herbicide from the prenatal period to the end of an experiment were evaluated. The experiment used 90 male and female, Wistar, aged to 10 weeks rats, divided into two groups: I-control-30 and II-60 animals which received the water solution of 2,4-D acid sodium salt in a daily dose of 250 mg/kg b.w. It was given with drinking water every day. The animals were sacrificed after 24 hours, 4, 6, and 10 weeks of the experiment. The results obtained showed that the administration of 2,4-D acid to rats in the prenatal and postnatal period, in a dose inducing subacute intoxication leads to histological and histochemical changes in the liver. The observed changes indicate disorders in energetic processes in hepatocytes and are morphological exponents of detoxicative processes there. They are most intensified with newborn rats. It suggest also, the pregnants ought not to work with 2,4-D and should avoid any contact with herbicides belongs to the 2,4-dichlorophenoxyacetic acid group.

Upham BL, Boddy B, Xing XS, Trosko JE, Masten SJ. 1997 Oct. Non-genotoxic effects of selected pesticides and their disinfection by-products on gap junctional intercellular communication. Ozone-Science & Engineering 19(4):351-369. Abstract: The presence of pesticides in drinking water poses a human health risk and disinfection can either exacerbate or reduce these health risks by producing by-products that are either more or less toxic than the parent compound. Human exposure to environmental pollutants usually is at low levels in nature, chronic, and often involves non-genotoxic mechanisms of cellular injury such as aberrant intercellular gap junctional communication (GJIC). Compounds toxic to GJIC are known to cause tumor promotion, neuropathy and teratogenesis. The non-genotoxic effects of atrazine, alachlor, carbofuran, 2,4-D and lindane and their ozonation and chlorination by-products were determined as a function of gap junction intercellular communication (GJIC) activity in F344-WB rat liver epithelial cells. Lindane did not react with either ozone or chlorine and was toxic to GJIC. All the other pesticides reacted with both ozone and chlorine to various degrees and the by-products formed from these pesticides were not significantly more toxic to GJIC than the parent compounds.

Rosso SB, Di Paolo OA, Evangelista de Duffard AM , Duffard R. 1997 Sep 19. Effects of 2,4-dichlorophenoxyacetic acid on central nervous system of developmental rats. Associated changes in ganglioside pattern. Brain Res 769(1):163-7 . Abstract: Neonate rats were treated with 2,4-dichlorophenoxyacetic acid (2,4-D) from the 7th or 12th until the 17th or 25th postnatal day. Two drug dosages were used: 70 and 100 mg/kg body weight of 2,4-D. At the 17th day of age, no changes were observed in body weight, protein and DNA content. However, 25-day-old treated pups showed diminutions in body and brain weight, protein and DNA levels, depending on doses and period of treatment. With respect to ganglioside levels, few changes were observed in treated animals until the 17th day of age. However, at the 25th day, with higher dose and longer treatment a diminution in all parameters analyzed was observed. These results suggest a delay in CNS development when pups were exposed to a very severe chemical injury with 2,4-D. On the other hand, when the chemical injury was not too severe, the brain would be capable to trigger biochemical mechanisms producing a plasticity response which is expressed as changes in ganglioside content and composition.

Sastry BV, Janson VE, Clark CP, Owens LK. 1997 Jun. Cellular toxicity of 2,4,5-trichlorophenoxyacetic acid: formation of 2,4,5-trichlorophenoxyacetylcholine. Cell Mol Biol (Noisy-Le-Grand) 43(4):549-57.Abstract: One of the toxic symptoms of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) is reduction in metabolic rate and subsequent growth retardation. Acetylcholine (ACh) serves as an essential growth factor to facilitate amino acid transport and to promote fetal growth. Hydatidiform mole lacks the capacity for synthesis of ACh, and inhibition of ACh synthesis depresses placental amino acid transport. Therefore, we studied the formation of 2,4,5-acetylcoenzyme A (2,4,5-T-CoA) by acetylcoenzyme A synthase (ACoAS) and the formation of 2,4,5-T-ACh by human placental choline acteyltransferase (ChA) from 2,4,5-T-CoA and choline. In these studies, the widely used analog of 2,4,5-T as an herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), was also included. These studies have the following results (M +/- S.D.; N,6):1) The enzymatic rates of formation of acetyl-CoA, 2,4,5-T-CoA, and 2,4-D-CoA by ACoAS were 32 +/- 4, 23 +/- 3 and 26 +/- 8 nmol/mg protein/5 min., respectively; 2) There were no significant differences in the maximal amounts (nmol/mg protein) of acetyl-CoA (128 +/- 4), 2,4,5-T-CoA (125 +/- 8) and 2,4-D-CoA (96 +/- 6) formed during the reaction period of 50 min.; 3) 14C-2,4-ACh was formed from 14C-2,4-D-CoA and choline by placental-ChA; 4) Low concentrations (EC50 1-2 microM) of synthetic 2,4,5-T-ACh and 2,4-D-ACh decreased the contraction heights of the rat phrenic nerve-hemidiaphragm when the nerve or the muscle was electrically stimulated, and 5) Similar results were obtained with 2,4,6-T-ACh, an analog of 2,4,5-T-ACh. These observations indicate that chlorophenoxyherbicides form false cholinergic messengers in the nerve, muscle and placenta. These false cholinergic messengers can be formed at both muscarinic and nicotinic synaptic sites and also in non-neuronal cells, where ACh plays an important regulatory role as a local hormone, and act as blocking agents. These results will partially explain myotonia, ventricular fibrillation and fetal growth retardation induced by these herbicides.

Sever LE, Arbuckle TE, Sweeney A. 1997 Apr-Jun. Reproductive and developmental effects of occupational pesticide exposure: the epidemiologic evidence. Occup Med 12(2):305-25.Abstract: There is increasing evidence for reproductive and developmental effects of both maternal and paternal pesticide exposures. Here is a summary of the epidemiologic data, culled from studies in humans, with significant attention to Agent Orange.

Brusco A, Saavedra JP, Garcia G, Tagliaferro P, Evangelista de Duffard AM, Duffard R. 1997 Apr. 2,4-dichlorophenoxyacetic acid through lactation induces astrogliosis in rat brain. Mol Chem Neuropathol 30(3):175-85.Abstract: Comparison of astroglial immunoreactivity in mesencephalon, cerebellum, and hippocampus of 25-d-old rat pups exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) through the mother's milk was made using a quantitative immunohistochemical analysis. A glial reaction was detected at the level of serotonergic nuclei and extreme astrogliosis in the hippocampus and cerebellum. A quantitative analysis of reactive astrocytes was performed by using GFAP and S-100 protein as specific markers. The study showed a significant increase in their number, size, number of processes, and density of immunostaining in 2,4-D-exposed animals. Exposure to 2,4-dichlorophenoxyacetic acid on the first days of life modifies the astroglial cytoarchitecture in parallel to previously described neuronal changes.

Duffard R, Garcia G, Rosso S, Bortolozzi A, Madariaga M, di Paolo O, Evangelista de Duffard AM. 1996 Nov-Dec. Central nervous system myelin deficit in rats exposed to 2,4-dichlorophenoxyacetic acid throughout lactation. Neurotoxicol Teratol 18(6):691-6.Abstract: Our results show that 2,4-dichlorophenoxyacetic acid (2,4-D) exposure through mother's milk during the period of rapid myelination (from the 15th to the 25th postnatal days) results in a myelin deficit in the pup's brain and demonstrates the vulnerability of the developing central nervous system (CNS) to 2,4-D. After 100 mg/kg 2,4-D administration to dams, brains of male and female rats show a significant diminution of myelin markers such as monohexosylceramide as well as phospholipids and free fatty acids (FFA) and an increase of cholesteryl esters. Histological studies revealed myelin deficit in some brain regions after 2,4-D treatment. These data indicate that 2,4-D, through the mother's milk, alters the myelination process during a specific postnatal period.

Ha MC, Cordier S, Bard D, Le TB, Hoang AH, Hoang TQ, Le CD, Abenhaim L, Nguyen TN. 1996 Sep-Oct. Agent orange and the risk of gestational trophoblastic disease in Vietnam. Arch Environ Health 51(5):368-74.Abstract: There have been claims of an increased risk for gestational trophoblastic disease (i.e., hydatidiform mole and choriocarcinoma) in Vietnam since the period of Agent Orange sprayings. In 1990, we conducted a case-control study in Ho Chi Minh City to investigate risk factors for gestational

trophoblastic disease in Vietnam. Eighty-seven married women, all of whom had a recent pathologic diagnosis of gestational trophoblastic disease, identified in the Obstetrical and Gynecological Hospital, were included in the study. Eighty-seven married women who were admitted mainly in the surgery departments of the same hospital were the controls, and they were matched to cases for age and area of residence. Odds ratios (ORs), adjusted for matching variables and other potential confounders, were estimated with unconditional logistic regression. A statistically significant trend in risk was observed with previous live births (p = .01). Cases were found to eat less meat per wk (OR = 0.4, 95% confidence interval [95% CI] = 0.2-0.9 for > or = five meat dishes) and to own fewer consumer goods than controls. An increase in risk was associated with the breeding of pigs (OR = 5.7, 95% CI = 1.2-27.6 for raising three or more pigs). A cumulative Agent Orange exposure index was constructed, using the patient's complete residence history. No significant difference was found between cases and controls for this index (OR = 0.7, 95% CI = 0.2-1.8 for high-exposure category), nor was such a difference noted for the agricultural use of pesticides.

Morgan MK, Scheuerman PR, Bishop CS, Pyles RA. 1996 Jun 7. Teratogenic potential of atrazine and 2,4-D using FETAX. J Toxicol Environ Health 48(2):151-68.Abstract: The teratogenic potential of commercial formulations of atrazine (40.8%) and 2,4-D was evaluated using FETAX (frog embryo teratogenic assay--Xenopus). Because these herbicides have been detected in ground and surface water, this study was designed to determine the adverse effects in buffer and natural water for both herbicides. All treatments showed a significant concentration-response effect on exposed embryos, except for the 2,4-D natural water sample. Atrazine (solubility of the commercial formula used 70 mg/L at 20 degrees C), compared to 2,4-D (solubility = 311 mg/L at pH = 1 and 25 degrees C), had a significantly greater teratogenic effect in both the buffer (atrazine EC50 = 33 mg/L, LC50 = 100 mg/L, TI = 3.03; 2,4-D EC50 = 245 mg/L, LC50 = 254 mg/L, TI = 1.04) and natural water samples (atrazine EC50 < 8 mg/L, LC50 = 126 mg/L; 2,4-D EC50 and LC50 > 270 mg/L). The 2,4-D EC50 and LC50 values for the buffer were similar at 245 mg/L and 254 mg/L. These similar values and the teratogenic index (TI) of 1.04 suggested that 2,4-D was more embryotoxic than teratogenic to frog embryos at high concentrations. Atrazine in natural water demonstrated a significantly greater EC50 (100% abnormality at 8 mg/L, the lowest test concentration) to frog embryos than the buffer experiment (EC50 = 33 mg/L). The extrapolated lowest observable adverse effect concentration (LOAEC) for the natural water experiment was 1.1 mg/L. These results suggest that atrazine toxicity is enhanced by the synergistic or additive effects of some component of the water or atrazine was already present in the sample. In contrast to atrazine, 2,4-D was less toxic in natural water than buffer. These results suggest that both atrazine and 2,4-D pose little threat, since their embryotoxicity and teratogenicity to frog embryos occur at high concentrations approaching their maximum solubility levels in water.

Stephenson J. 1996 Apr 10. New IOM report links agent orange exposure to risk of birth defect in Vietnam vets' children. JAMA 275(14):1066-7.

Sulik M, Matus A, Musiatowicz B, Sulkowska M, Kemona A, Kisielewski W, Sobaniec-Lotowska M, Barwijuk-Machala M. 1996. The effect of a herbicide--sodium salt of 2,4-dichlorophenoxyacetic acid on guerin carcinoma. Rocz Akad Med Bialymst 41(2):347-62.Abstract: The effect of sodium salt of 2,4-dichlorophenoxyacetic acid, being an active component of herbicide "PIELIK", upon the development of Guerin carcinoma implanted in male Wistar rats, was studied. 192 animals were divided in to 6 equal groups: I-animals which obtained physiological salt solution; II-rats exposed to the herbicide in postlactational period; III-animals with Guerin carcinoma, non exposed to the herbicide; IV- rats exposed to the herbicide in postlactational period+Guerin carcinoma; V-animals exposed to the herbicide from prenatal period to the end of an experiment, without Guerin carcinoma; VI-the same as in V group, but with Guerin carcinoma. The effect of the herbicide on tumor growth dynamism (diameters and mass), degree of tumour malignancy (metastases to lymph nodes), animals survival time and morfological changes in the primary tumour and in metastases was evaluated. Basing of the results obtained, it was stated that this herbicide accelerates the development of Guerin carcinoma and reduces the survival time in the rats exposed to it in the prenatal and postnatal period. However, it does not significantly influence the growth of the carcinoma in the rats exposed only in the postlactational period.

Kornuta N, Bagley E, Nedopitanskaya N. 1996. Genotoxic effects of pesticides. J Environ Pathol Toxicol Oncol

15(2-4):75-8. Abstract: Epidemiologic data showed an increase in the number of cancer cases in persons involved in agricultural production using pesticides. According to IARC, more than 25% of pesticides are classified as oncogens. In recent years, the concept of malignant tumors developing after environmental contamination with chemicals has been accepted. Changes in genetic material are at the basis of this process because many environmental pollutants are chemical carcinogens and mutagens with the capacity of causing DNA damage. DNA damage was proposed as a useful parameter for assessing the genotoxic properties of environmental pollutants. The correlation between exposure to carcinogenic substance and the level of DNA damage is essential. Pesticides are highly biologically active chemicals. They may interact with DNA and damage its structure. Such interaction may be critical for the manifestation of carcinogenic properties of different chemicals. We report on the organotropic genotoxic effects of different chemical classes of pesticides (decis, cypermetrin, 2,4-D, polyram) studied by means of alkaline unwinding assay DNA.

Burroughs BL, Johnson CS, Garry VF. 1996. In vitro micronucleus response of commercial chlorophenoxy herbicides and adjuvants:1-5. Abstract: (Rough draft without graphs) Chlorophenoxy herbicides, particularly 2,4-D have been epidemiologically associated with excess Non Hodgkins Lymphoma in some studies while not in others (1,2,3,4). In vivo and in vitro studies in animals or in cultured cells of chemically pure chlorophenoxy herbicide do not suggest that these herbicides are notably genotoxic (1 ibid., 5,6,7,8). On the other hand, adjuvants sometimes used in conjunction with these herbicides as spreading and sticking agents have not to our knowledge been examined for genotoxic potential. To test the hypothesis that contaminants in these herbicides or adjuvants might have genotoxic potential, commercial grade chlorophenoxy herbicides, other herbicides and adjuvants were studied. Chemicals used in these in vitro studies were obtained from forest pesticides applicators who use these products in their work. This report is part of a larger laboratory based human population study of forest pesticide applicators.

Evangelista de Duffard AM, Brusco A, Duffard R , Garcia G, Pecci Saavedra J. 1995 Oct. Changes in serotonin-immunoreactivity in the dorsal and median raphe nuclei of rats exposed to 2,4-dichlorophenoxyacetic acid through lactation. Mol Chem Neuropathol 26(2):187-93. Abstract: Comparison of serotonin-immunoreactive (SER-IR) neurons in nucleus raphe dorsalis (NRD) and median raphe nucleus (MRN) of 25-d-old rat pups exposed to 70 mg/kg/d 2,4-dichloro-phenoxyacetic acid through mothers milk and control pups was made using an immunohistochemical analysis. Significant 2,4-D-treatment-related increase in size and density of SER-IR neuronal somata as well as in fiber length were observed. We postulate that exposure to 2,4-dichlorophenoxyacetic acid on the first day of life would modify the synthesis of 5-HT or the maturation of the brain serotonergic system.

Wolfe WH, Michalek JE, Miner JC, Rahe AJ, Moore CA, Needham LL, Patterson DG Jr. 1995 Jan. Paternal serum dioxin and reproductive outcomes among veterans of Operation Ranch Hand. Epidemiology 6(1):17-22. Abstract: We studied whether paternal exposure to Agent Orange and its dioxin contaminant (2,3,7,8-tetrachlorodibenzo-p-dioxin) during the Vietnam War is related to adverse reproductive outcomes after service in Southeast Asia. The index cohort comprises conceptions and children of veterans of Operation Ranch Hand, the unit responsible for aerial spraying of herbicides in Vietnam from 1962 to 1971. The comparison cohort comprises conceptions and children of Air Force veterans who served in Southeast Asia during the same period but who were not involved with spraying herbicides. We found no meaningful elevation in risk for spontaneous abortion or stillbirth. In analyses of birth defects, we found elevations in risk in some organ system categories, which, after review of the clinical descriptions, were found to be not biologically meaningful. There was an increase in nervous system defects in Ranch Hand children with increased paternal dioxin, but it was based on sparse data. We found no indication of increased birth defect severity, delays in development, or hyperkinetic syndrome with paternal dioxin. These data provide little or no support for the theory that paternal exposure to Agent Orange and its dioxin contaminant is associated with adverse reproductive outcomes.

[Anonymous]. 1993. PesticidesSchardein JL. Chemically induced birth defects. second ed. New York: Marcel Dekker Inc. p 675-721 (Ch. 25). Abstract: Pesticides occupy a rather unique position among the many chemicals that humans encounter in that they are deliberately added to the environment for the purpose of injuring or killing some form of life: "pests" in this connotation. The term pesticide is a general term and includes a variety of chemicals with different uses. As a group, for purposes here, they include

insecticides, herbicides, and fungicides. A 1982 Congressional report estimated that 60-70% of the pesticides registered for use had not been adequately tested for their ability to cause birth defects.

Munro IC, Carlo GL, Orr JC, Sund KG, Wilson RM, Kennepohl E, Lynch BS, Jablinske M, Lee NL. 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-d [review]. Journal of the American College of Toxicology 11(5):559 ff. Abstract: The safety of 2,4-D to farm and forestry workers, commercial applicators and the general public has been of continuing concern because certain epidemiological studies of groups potentially exposed to 2,4-D have suggested a relationship between 2,4-D use and increased risk of soft tissue sarcoma, Hodgkin's disease or non-Hodgkin's lymphoma. This review on 2,4-D is unique in that the approach taken was to integrate data from worker exposure studies, whole animals, metabolic and other relevant laboratory studies with the epidemiological findings to assess the extent to which there is scientific support for the hypothesis that 2,4-D exposure is associated with any increased risk of human cancer. The case-control epidemiological studies that have been the source of the cancer risk hypothesis are inconclusive. Problems in assessing exposure based on patients' memories make these studies difficult to interpret. Cohort studies of exposed workers do not generally support the specific hypothesis that 2,4-D causes cancer. Taken together, the epidemiological studies provide, at best, only weak evidence of an association between 2,4-D and the risk of cancer. Importantly, the cancer hypothesis is not supported by other data. A critical evaluation of the exposure data indicates that exposure to 2,4-D in user groups is intermittent and much lower than the doses tested chronically in long-term animal studies that have not shown evidence of tumor induction. Moreover, the structure of 2,4-D does not suggest it would be a carcinogen. 2,4-D is a simple organic acid, that is largely excreted unchanged, and there is no evidence that it is metabolized to critically reactive metabolites or accumulates in tissues. This evidence is supported by a large body of negative studies on genotoxicity, which taken together with the metabolic studies, clearly indicates that 2,4-D is highly unlikely to be a genotoxic carcinogen. Furthermore, 2,4-D has no known hormonal activity and does not induce proliferative changes in any tissue or organ, indicating that it does not possess any of the characteristics of non-genotoxic animal carcinogens. Thus the available mechanistic studies provide no plausible basis for a hypothesis of carcinogenicity. In this review, data relating to potential neurotoxicity, immunotoxicity and reproductive toxicity also were evaluated. There is no evidence that 2,4-D adversely affects the immune system and neurotoxic and reproductive effects only have been associated with high toxic doses that would not be encountered by 2,4-D users. Historical exposures to 2,4-D by user groups, particularly farmers, forestry workers and commercial applicators, would be higher than those sustained under present rigorous standards for application which involve the use of protective clothing and other measures to reduce exposure. Proposed label changes indicate that in the future exposures will be even further reduced. Viewed in this context, the available data indicate that the potential public health impact of 2,4-D, including the risk of human cancer, was negligible in the past and would be expected to be even smaller in the present and future.

Moses M. 1992. Pesticides. In. Public Health & Preventive Medicine. 13th ed. p 479-489 (Ch. 24). (Last, Wallace.

Jacobi H, Metzger J, Witte I. 1992. Synergistic effects of Cu(II) and dimethylammonium 2,4-dichlorophenoxyacetate (U46 D fluid) on PM2 DNA and mechanism of DNA damage. Free Radic Res Commun 16(2):123-30. Abstract: Dimethylammonium 2,4-dichlorophenoxyacetate (2,4-D . DMA) induced strand breaks in PM2 DNA when incubated with CuCl2, whereas 2,4-D . DMA alone or CuCl2 alone did not show any or only a negligible effect. The formation of single strand breaks increased linearly with time and concentration of 2,4-D . DMA. Neocuproine, a specific Cu(I) chelator totally prevented strand break formation. So did catalase (up to 100 mM 2,4-D . DMA), but DMSO had only a small protective effect. 2,4-Dichlorophenol, CO2 and formaldehyde were detected as reaction products of 2,4-D and CuCl2. From these results a redox reaction of Cu(II) and 2,4-D is proposed, which could explain the DNA damaging properties of CuCl2/2,4-D . DMA.

Ibrahim MA, Bond GG, Burke TA, Cole P, Dost FN, Enterline PE, Gough M, Greenberg RS, Halperin WE, McConnell E, et al. 1991 Dec. Weight of the evidence on the human carcinogenicity of 2,4-D. Environ Health Perspect 96:213-22. Abstract: The phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is widely used to control the growth of weeds and broadleaf plants. We convened a panel of 13 scientists to weigh the evidence on the human carcinogenicity of 2,4-D. The panel based its findings on a review of the

toxicological and epidemiological literature on 2,4-D and related phenoxy herbicides. The toxicological data do not provide a strong basis for predicting that 2,4-D is a human carcinogen. Although a cause-effect relationship is far from being established, the epidemiological evidence for an association between exposure to 2,4-D and non-Hodgkin's lymphoma is suggestive and requires further investigation. There is little evidence of an association between use of 2,4-D and soft-tissue sarcoma or Hodgkin's disease, and no evidence of an association between 2,4-D use and any other form of cancer. Scientists on the panel were asked to categorize 2,4-D as a "known," "probable," "possible," or "unlikely" carcinogen or as a noncarcinogen in humans. The predominant opinion among the panel members was that the weight of the evidence indicates that it is possible that exposure to 2,4-D can cause cancer in humans, although not all of the panelists believed the possibility was equally likely: one thought the possibility was strong, leaning toward probable, and five thought the possibility was remote, leaning toward unlikely. Two panelists believed it unlikely that 2,4-D can cause cancer in humans.

Sanner T, Mikalsen SO, Rivedal E. 1991. Hepatic peroxisome proliferators induce morphologic transformation of Syrian hamster embryo cells, but not peroxisomal beta oxidation. Prog Clin Biol Res 369:77-89.

de Duffard AM, de Alderete MN, Duffard R. 1990 Dec 3. Changes in brain serotonin and 5-hydroxyindolacetic acid levels induced by 2,4-dichlorophenoxyacetic butyl ester. Toxicology 64(3):265-70. Abstract: Brain concentrations of Serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) were determined in male, mother and virgin female adult rats after exposure to 69 mg/kg body weight/day of 2,4-dichlorophenoxyacetic butyl ester (2,4-Dbe) during 15 or 45 consecutive days. Both 5-HT and 5-HIAA concentrations were increased in the brain. These effects reverted to levels even lower than controls, when the animals were fed an untreated diet after the 2,4-Dbe treatment. High 5-HT and 5-HIAA brain concentrations were also observed in adult rats born from treated mothers (during pregnancy and lactancy) and fed with or without treated diet after weaning. Two different effects on serotoninergic system were detected: a transient effect if 2,4-Dbe was given to adult rats in a short period of time and a permanent effect if the herbicide was supplied during pre- and post birth period (rat brain development). However, in utero exposed but lactationally cross-fostered rat pups were not affected, suggesting that prenatal exposure did not have any influence on the postnatal status of the neurotransmitter(s).

Chernoff N, Setzer RW, Miller DB, Rosen MB, Rogers JM. 1990 Dec. Effects of chemically induced maternal toxicity on prenatal development in the rat. Teratology 42(6):651-8. Abstract: The hypothesis that chemically induced overt maternal toxicity induces a characteristic syndrome of adverse developmental effects in the rat was investigated. Pregnant animals (Sprague-Dawley strain) were dosed by oral gavage with one of a series of compounds on days 6-15 of gestation. These chemicals were diquat (DIQ), ethylene-bis-isothiocyanate (EBIS), toxaphene (TOX), styrene (STY), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenol (2,4,5-Tr), triphenyl tin hydroxide (TPTH), and cacodylic acid (CAC). The compounds were chosen because they exhibited little or no developmental toxicity in previous studies. Dosage levels producing maternal weight loss and/or lethality were determined from preliminary toxicity studies. Significant maternal weight reductions were noted during the course of treatment with all compounds except CAC and 2,4,5-Tr. Maternal lethality was produced by EBIS, TOX, 2,4,-D, and 2,4,5-Tr. The main treatment-related developmental toxicity noted in litters at term consisted of increased lethality (EBIS, TPTH) and decreased fetal weight (EBIS and CAC). Treatment-related anomalies were seen in litters treated with 2,4-D and TOX (supernumerary ribs) and with EBIS and STY (enlarged renal pelvis). No significant developmental effects were produced with DIQ, or 2,4,5-Tr. This study indicates that overt maternal toxicity as defined by weight loss or mortality is not always associated with the same defined syndrome of adverse developmental effects in the rat.

Schop RN, Hardy MH, Goldberg MT. 1990 Nov. Comparison of the activity of topically applied pesticides and the herbicide 2,4-D in two short-term in vivo assays of genotoxicity in the mouse. Fundam Appl Toxicol 15(4):666-75. Abstract: Genotoxicity of eight topically applied compounds was determined using the bone marrow micronucleus (MN) test and hair follicle nuclear aberration (NA) assay in CD1 mice. Twenty-four hours after a single treatment, cyclophosphamide (CY), applied at doses corresponding to 1/4, 1/8, 1/16, and 1/32 of the published dermal LD50, and N-methyl-N-nitrosourea (MNU), applied at 1/4, 1/8, and 1/16 of the published dermal LD50, were found to increase the incidence of NA in a dose-dependent manner. The frequency of MN was significantly increased only at the highest dose of CY. Using the same protocol,

six pesticides applied in dimethyl sulfoxide (DMSO) at doses of 1/8, 1/16, and 1/32 of the dermal LD50 were investigated. Aminocarb and chlordane induced a dose-dependent increase in the frequency of NA, while there was an observed increase in NA incidence at only the highest doses of dichlorvos (DDVP), 4,4'-DDT (DDT), and 2,4-dichlorophenoxyacetic acid (2,4-D). No effect was observed with fenitrothion on nuclear aberrations in hair follicles. Except for the highest dose of chlordane, none of the pesticides tested positive in the bone marrow micronucleus test. Serum cholinesterase levels were reduced to 70 +/- 4.7% of the DMSO control level with DDVP, 57 +/- 8.2% with aminocarb, and 60.3 +/- 4.8% with fenitrothion, indicating some systemic activity with these topically applied agents. The data suggest that aminocarb, chlordane, DDVP, DDT, and 2,4-D are genotoxic as determined by the NA assay and that this assay may be more useful in detecting topically applied genotoxic agents than the more often used bone marrow micronucleus test.

Mikalsen SO, Ruyter B, Sanner T. 1990 Feb 1. Effects of hepatic peroxisome proliferators and 12-O-tetradecanoyl phorbol-13-acetate on catalase and other enzyme activities of embryonic cells in vitro. Biochem Pharmacol 39(3):527-35.[1][SEP]Abstract: The effects of the hepatic peroxisome proliferators (HPPs) clofibrate, di-(2-ethylhexyl)-phthalate (DEHP), mono-(2-ethylhexyl)phthalate (MEHP) and 2,4-dichlorophenoxy acetic acid (2,4-D) on the activities of some peroxisome-associated enzymes and marker enzymes for other organelles, have been studied in primary Syrian hamster embryo (SHE) cells and Wistar rat embryo (WRE) cells. The majority of the cells are fibroblast-like. 12-O-Tetradecanoyl phorbol-13-acetate (TPA) was included as it has been suggested that it may act as a peroxisome proliferator. The specific activities of catalase, fatty acyl-CoA oxidase (FAO) and peroxisomal beta-oxidation were approximately 100-fold lower in the embryonic cells than in rat hepatocytes. Other peroxisome-associated oxidases were not detected. The dihydroxyacetone-phosphate acyltransferase (DHAPAT) activity was comparable to that in rat liver. Marker enzymes for other organelles had specific activities comparable to rat hepatocytes. Catalase was shown by digitonin titration to be contained in a peroxisome-like compartment in both SHE and WRE cells. Clofibrate, DEHP and MEHP increased the catalase activity, which might suggest peroxisome proliferation. However, the findings that FAO and peroxisomal beta-oxidation did not increase or only very slightly, argue against peroxisome proliferation. 2,4-D and TPA induced no or only a very slight increase in the catalase activity.

Mikalsen SO, Holen I, Sanner T. 1990 Jan. Morphological transformation and catalase activity of Syrian hamster embryo cells treated with hepatic peroxisome proliferators, TPA and nickel sulphate. Cell Biol Toxicol 6(1):1-13.[1][SEP]Abstract: The abilities of the hepatic peroxisome proliferators (HPPs) clofibrate, di(2-ethylhexyl)phthalate (DEHP), mono(2-ethylhexyl)-phthalate (MEHP), 2,4-dichlorophenoxy acetic acid (2,4-D), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and tiadenol to induce morphological transformation and to increase the catalase activity of Syrian hamster embryo (SHE) cells were studied. DEHP, MEHP, clofibrate and tiadenol induced morphological transformation of SHE cells and increased the catalase activity. DEHP was more potent than clofibrate and tiadenol in both inducing catalase and morphological transformation, while MEHP seemed more potent than DEHP in inducing catalase, but not morphological transformation, 2,4,5-T and 2,4-D did not induce morphological transformation, but 2,4,5-T was more potent than clofibrate in increasing the catalase activity. These results show that several HPPs induce morphological transformation of SHE cells and an increase in the catalase activity. There is, however, no direct connection between these two parameters, as seen from the results of 2,4,5-T. The tumor promoter TPA, and the metal salt nickel sulphate, induced morphological transformation of SHE cells without any appreciable increase in the catalase activity. These results further corroborate the dissociation between induction of morphological transformation and the increase in catalase activity.

Robinowitz R, Roberts WR, Dolan MP, Patterson ET, Charles HL, Atkins HG, Penk WE. 1989 Sep. Carcinogenicity and teratogenicity vs. psychogenicity: psychological characteristics associated with self-reported Agent Orange exposure among Vietnam combat veterans who seek treatment for substance abuse. J Clin Psychol 45(5):718-28.[1][SEP]Abstract: This study asked, "What are the psychological characteristics of Vietnam combat veterans who claim Agent Orange exposure when compared with combat-experienced cohorts who do not report such contamination?" The question was researched among 153 heroin addicts, polydrug abusers, and chronic alcoholics who were seeking treatment: 58 reported moderate to high defoliant exposure while in combat; 95 reported minimal to no exposure while in Vietnam. The null hypothesis was accepted for measures of childhood and present family social climate, premilitary

backgrounds, reasons for seeking treatment, patterns and types of illicit drug and alcohol use, interpersonal problems, intellectual functioning, and short-term memory. The null hypothesis was rejected for personality differences, however, those who self-reported high Agent Orange exposure scored significantly higher on MMPI scales F, Hypochondriasis, Depression, Paranoia, Psychasthenia, Schizophrenia, Mania, and Social interoversion. The results suggest that clinicians carefully assess attributional processing of those who report traumatic experience.

Blakley PM, Kim JS, Firneisz GD. 1989 Jun. Effects of preconceptional and gestational exposure to Tordon 202c on fetal growth and development in CD-1 mice. Teratology 39(6):547-53. Abstract: Female CD-1 mice were exposed to Tordon 202c (a picloram and 2,4-D combination herbicide) in the drinking water at concentrations of 0.21, 0.42, and 0.84% for 60 days prior to mating with untreated males. One-half of the pregnant females subsequently continued treatment throughout gestation while the remaining females were maintained on distilled water. Fetal weight, crown-rump length, placental weight, and maternal gestational weight gain were reduced in a dose-dependent manner following combined preconceptional and gestational exposure. The incidence of malformed fetuses (cleft palate, renal agenesis, hydronephrosis, unilateral testicular agenesis, and umbilical hernia) and fetuses with variants (especially incomplete ossification of the skeleton) were increased in a dose-dependent manner following combined exposure. Increased maternal mortality and decreased preconception weight gain were observed in the highest-dosage group. Relative maternal liver weight was increased in a dose-dependent manner. The results suggest that combined preconceptional and gestational exposure to Tordon 202c is required for teratogenesis and fetal growth depression. Preconceptional exposure alone is not effective in increasing the risk for embryotoxicity.

[Anonymous]. 1989 Jun. NTP Toxicology and Carcinogenesis Studies of 2,4-Dichlorophenol (CAS No. 120-83-2) in F344/N Rats and B6C3F1 Mice (Feed Studies). Natl Toxicol Program Tech Rep Ser 353:1-182. Abstract: 2,4-Dichlorophenol is a chemical intermediate used principally in the manufacture of the herbicide 2,4-dichlorophenoxyacetic acid. Toxicology and carcinogenesis studies were conducted by feeding diets containing 2,4-dichlorophenol (greater than 99% pure) for 14 days, 13 weeks, or 2 years to groups of F344/N rats and B6C3F1 mice of each sex. Genetic toxicology tests were conducted in Salmonella typhimurium, mouse L5178Y lymphoma cells, and Chinese hamster ovary (CHO) cells. Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, male and female rats and mice were given diets containing 2,4-dichlorophenol at concentrations up to 40,000 ppm. One high dose male mouse died before the end of the studies; no deaths occurred in any other group, and no compound-related lesions were seen at necropsy in rats or mice. In the 13-week studies, groups of 10 rats and 10 mice of each sex were fed diets containing 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm 2,4-dichlorophenol. All rats lived to the end of the studies, whereas all mice that received 40,000 ppm died during the first 3 weeks of the studies. Final mean body weights of rats that received 20,000 or 40,000 ppm and of male mice that received 20,000 ppm were at least 10% lower than those of controls. Bone marrow atrophy in rats and necrosis and syncytial alteration (multinucleated hepatocytes) in the liver of male mice were compound-related effects. Two-year studies were conducted by feeding diets containing 0, 5,000, or 10,000 ppm 2,4-dichlorophenol to groups of 50 male rats and 50 male and 50 female mice for 103 weeks. Groups of 50 female rats received diets containing 0, 2,500, or 5,000 ppm. Body Weight and Survival in the Two-Year Studies: Mean body weights of high dose male and female rats, high dose male mice, and both dosed groups of female mice were generally lower than those of controls. No significant differences in survival were observed between any groups of rats or mice of either sex (male rats: control, 33/50; low dose, 25/50; high dose, 32/50; female rats: 34/50; 43/50; 40/50; male mice: 33/50; 32/50; 31/50; female mice: 45/50; 40/50; 43/50). The average daily feed consumption by rats in the low dose and high dose groups was 94%-97% that by the controls. The estimated daily mean consumption of 2,4-dichlorophenol was 210 or 440 mg/kg for low dose or high dose male rats and 120 or 250 mg/kg for low dose or high dose female rats. The average daily feed consumption by mice in the low dose and high dose groups was 97% and 78% of that by the controls for males and 94% and 85% for females. The estimated daily mean consumption of 2,4-dichlorophenol was 800 or 1,300 mg/kg for low dose or high dose male mice and 430 or 820 mg/kg for low dose or high dose female mice. Nonneoplastic and Neoplastic Effects in the Two-Year Studies: There were no compound-related increased incidences of neoplastic lesions in rats or mice. The incidence of mononuclear cell leukemia was decreased in dosed male rats relative to that in controls (control, 31/50; low dose, 17/50; high dose, 17/50); the incidence of malignant lymphomas was decreased in high dose female mice (4/50) relative to that in controls (12/50). Syncytial alteration of hepatocytes was observed at

increased incidences in dosed male mice (11/50; 33/49; 42/48). Genetic Toxicology: The mutagenic effect of 2,4-dichlorophenol in S. typhimurium strain TA1535 was considered to be equivocal only in the presence of hamster S9; 2,4-dichlorophenol produced no increases in revertant colonies in strains TA98, TA100, or TA1537 with or without exogenous metabolic activation. 2,4-Dichlorophenol increased trifluorothymidine (Tft) resistance in the mouse L5178Y assay without metabolic activation; it was not tested with activation. In cultured CHO cells, 2,4-dichlorophenol did not induce chromosomal aberrations but did significantly increase the frequency of sister chromatid exchanges (SCEs) both in the presence and absence of S9. Audit: The data, documents, and pathology materials from the 2-year studies of 2,4-dichlorophenol have been auom the 2-year studies of 2,4-dichlorophenol have been audited. The audit findings show that the conduct of the studies is documented adequately and support the data and results given in this Technical Report. Conclusions: Under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity for male F344/N rats fed diets containing 5,000 or 10,000 ppm 2,4-dichlorophenol or for female F344/N rats fed diets containing 2,500 or 5,000 ppm 2,4-dichlorophenol. There was no evidence of carcinogenic activity for male or female B6C3F1 mice fed diets containing 5,000 or 10,000 ppm 2,4-dichlorophenol. Synonyms: 2,4-DCP; 2,4-dichlorohydroxybenzene

Blakley PM, Kim JS, Firneisz GD. 1989 Mar. Effects of paternal subacute exposure to Tordon 202c on fetal growth and development in CD-1 mice. Teratology 39(3):237-41.[1]Abstract: Male CD-1 mice were exposed to Tordon 202c (a picloram and 2,4-D combination herbicide) in the drinking water at concentrations of 0.21, 0.42, and 0.84% solutions for 60 days prior to mating with untreated females. Subsequently there was no exposure to Tordon 202c during gestation. Fetal weight and crown-rump length were reduced in the highest dosage group. The incidence of malformed fetuses (e.g., ablepharon, cleft palate, and unilateral agenesis of the testes) was increased in the middle dosage group while the incidence of fetuses with variants was increased in the lowest (e.g., an extra pair of ribs) and the highest dosage groups (e.g., incomplete ossification of the skeleton). The frequency of pregnancy failure was increased in the middle dosage group. Indices of paternal toxicity included increased lethality and decreased water consumption in the highest dosage group and increased relative spleen weights in the lowest and middle dosage groups. The results suggest paternally mediated reproductive toxicity.

Blakley PM, Kim JS, Firneisz GD. 1989. Effects of gestational exposure to Tordon 202c on fetal growth and development in CD-1 mice. J Toxicol Environ Health 28(3):309-16.[1]Abstract: The teratogenic effects of Tordon 202c, a picloram and 2,4-D combination formulation, are unknown. Pregnant CD-1 mice were exposed to Tordon 202c in the drinking water at concentrations of 0.10, 0.21, and 0.42% from d 6 to 15 of gestation. Fetal growth parameters, including body weight and crown-rump length, were reduced in a dose-dependent manner, as was placental weight. The incidence of dead fetuses/resorptions and malformed fetuses (especially cleft palate) was increased in the highest dosage group. A subtle indication of maternal toxicity was noted in the highest dosage group as evidenced by decreased water consumption and increased relative liver weight. The present study suggests that Tordon 202c is embryotoxic and teratogenic in CD-1 mice when administered during organogenesis.

Mohammad FK, St Omer VE. 1988 Feb. Effects of prenatal exposure to 2,4-D/2,4,5-T mixture on postnatal changes in rat brain glutamate, GABA, protein, and nucleic acid levels. Bull Environ Contam Toxicol 40(2):294-300.

Adhikari N, Grover IS. 1988. Genotoxic effects of some systemic pesticides: in vivo chromosomal aberrations in bone marrow cells in rats. Environ Mol Mutagen 12(2):235-42.[1]Abstract: The genotoxic effects of five pesticides (benomyl, 2,4-D, dimecron, monocrotophos, and vitavax) were evaluated in the rat bone marrow cytogenetic assay. The spectrum of aberrations observed included chromatid breaks, chromatid fragments, ring chromosomes, dicentric chromosomes, and chromosome fragments. It was observed that 2,4-D, dimecron, and vitavax were clastogenic, but the results obtained with benomyl and monocrotophos were equivocal.

St Omer VE, Mohammad FK. 1987 Sep. Ontogeny of swimming behavior and brain catecholamine turnover in rats prenatally exposed to a mixture of 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acids. Neuropharmacology 26(9):1351-8.[1]Abstract: Rats exposed in utero on gestational days 6-15, to nonfetotoxic and grossly nonteratogenic mixtures (50 or 100 mg/kg) of 2,4-D/2,4,5-T as found in Agent

Orange (but without significant contamination with 2,3,7,8 tetrachloro-p-dioxin) manifested subtle developmental neurotoxicity. Maturation of swimming behavior was significantly delayed on postnatal day 7 in both treatment groups. The concentration of norepinephrine (NE) in whole brain was significantly increased on postnatal day 15 in both treatment groups, whereas the concentration of dopamine (DA) was increased on postnatal day 15 at 100 mg/kg. The turnover and efflux rate constant of DA in whole brain were significantly reduced whereas the turnover time increased on postnatal day 3. The efflux rate constant for NE decreased and the turnover time increased significantly on postnatal day 15 at 100 mg/kg. These data indicate the value of ontogenic assessment following exposure to small doses, which result in functional alterations in the absence of overt toxic signs.

Hatch MC, Stein ZA. 1987. Agent Orange and risks to reproduction: the limits of epidemiology. Teratog Carcinog Mutagen 7(4):423-4.

Erickson JD, Mulinare J. 1987. Agent orange and risks to reproduction: the limits of epidemiology. Teratog Carcinog Mutagen 7(2):197-200.

Giri AK. 1986 Nov. Mutagenic and genotoxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin, a review. Mutat Res 168(3):241-8.Abstract: 2,3,7,8-Tetrachlorodibenzo-p-dioxin is one of the most toxic and highly stable compounds known in the environment. Due to uncontrolled exothermic reactions during manufacture of 2,4,5-T and other chlorophenoxy compounds, TCDD is released in the environment. The importance of determining the hazards to human health from dioxin became apparent because of its severe adverse health effects. 'Agent Orange' which is a mixture of 2,4,5-T and 2,4-D has been extensively used during the late Vietnam War. In this review an attempt has been made to evaluate the mutagenic and genotoxic effects of 2,3,7,8-TCDD as known at present.

Garrett NE, Stack HF, Waters MD. 1986 Nov. Evaluation of the genetic activity profiles of 65 pesticides. Mutat Res 168(3):301-25.Abstract: We have previously reported the qualitative results of a major study on 65 pesticides (Waters et al., 1982). Dose information from this investigation (either lowest effective or highest ineffective dose tested) has now been incorporated into a computerized data management system. This report focuses on the qualitative profiles of genetic activity produced by these pesticides and our efforts to classify them according to their genotoxic effects and chemical structures. Three main categories may be distinguished based on the qualitative results: Category 1 pesticides were active in most of the in vitro and in vivo assays employed. These 9 compounds include the structurally similar organophosphate insecticides, acephate, demeton, monocrotophos and trichlorfon; the phthalimide fungicide analogues, captan and folpet; and the thiocarbamate herbicide analogues, diallate, sulfallate and triallate. The 26 Category 2 compounds demonstrated fewer positive results and may be subdivided into two parts, one of which contains 12 halogenated aromatic or heterocyclic ring compounds, including the phenoxy herbicides, 2,4-D, 2,4-DB and 2,4,5-T. The remaining part of Category 2 (14 compounds) consists of structurally similar organophosphate insecticides, azinphos-methyl, crotoxyphos, disulfoton, methyl parathion; three similar ethylenebisdithiocarbamate fungicides, maneb, mancozeb, and zineb; three similar pyrethroid insecticides, allethrin, chrysanthemic acid, and ethyl chrysanthemate; and four structurally diverse compounds, cacodylic acid, dinoseb, sec.-butylamine and benomyl. The third category of 30 pesticides gave negative results in all tests and represents structurally diverse compounds. Using the computerized profile matching methodology, from 2080 possible pairwise chemical combinations of the 65 pesticides, 20 statistically significant pairs were selected, 6 groups of pesticides were identified which were substantially similar to groups of pesticides we had formed previously (Waters et al., 1982) based on genetic activity and chemical structure. The matches showed excellent qualitative and, in most cases, excellent quantitative agreement. Hence it appears that specific patterns of test results present in the genetic activity profiles are related directly to chemical structure. Conversely, the data suggests that certain groups of compounds may be recognized by a well defined series of concordant tests results. As additional data is added, comparison of test results for new chemicals with existing data for known genotoxicants should aid in the evaluation of potential genetic health hazards.

Hall W. 1986 Sep 1. The Agent Orange controversy after the Evatt Royal Commission. Med J Aust 145(5):219-25.Abstract: The Evatt Royal Commission Report on the Use and Effects of Chemical Agents on Australian Personnel in Vietnam has authoritatively rejected the substantive claims that were made by the

Vietnam Veterans' Association of Australia about the adverse effects of exposure to phenoxy herbicides in Vietnam on the health of Vietnam veterans and their families. The Commission concluded that Vietnam veterans were not exposed to toxic levels of chemicals in Vietnam; that they are not at any increased risk of fathering children with birth defects, or contracting cancer; and that, although they have slightly higher rates of psychiatric disorder, heart disease, alcoholism and alcohol-related disease, these effects are unconnected with exposure to chemicals in Vietnam. The reasons for these findings deserve to be given the widest possible publicity. Only by doing so is there any prospect of dissolving the misapprehension that Vietnam veterans have been poisoned by herbicides.

Mohammad FK, St Omer VE. 1986 Sep-Oct. Behavioral and developmental effects in rats following in utero exposure to 2,4-D/2,4,5-t mixture. Neurobehav Toxicol Teratol 8(5):551-60. Abstract: Groups (G) of pregnant rats were gavaged with 1:1, 2,4-D/2, 4, 5-T mixture at 0 (GO), 50 (G50), 100 (G100) and 125 (G125) mg/day on gestational days 6 to 15. G100 and G125 dams gained significantly less weight during pregnancy and delivered fewer offspring than control. Gestational lengths, neonatal sex ratios, birth weights, and physical appearance at birth were unaffected by treatments. On postnatal day (PND) 1, G125 mortality was significantly increased. Except for a significant weight reduction in G125 on PND60, postnatal growth and maturation were unaffected by treatments. 2,4-D/2, 4, 5-T significantly delayed male and female surface righting (PND2-5), and negative geotaxis at 45 degrees angle (PND15-17) in all groups; olfactory discrimination (PND9-11), and negative geotaxis at 25 degrees (PND7-11) in G100 and G125. On PND9 negative geotaxis at 25 degrees were significantly delayed in G50 females. d-Amphetamine challenges significantly increased running wheel activity of G125 males on PND22 and 23. 2,4-D/2,4,5-T was behaviorally teratogenic at all dosage levels.

Mustonen R, Kangas J, Vuojolahti P, Linnainmaa K. 1986 Jul. Effects of phenoxyacetic acids on the induction of chromosome aberrations in vitro and in vivo. Mutagenesis 1(4):241-5. Abstract: The effects of phenoxyacetic acid herbicides were investigated on the induction of chromosome aberrations in human peripheral lymphocyte cultures in vitro and in lymphocytes of exposed workers in vivo. Pure 2,4-dichlorophenoxyacetic acid (2,4-D; 0.125, 0.150, 0.200 and 0.350 mM) did not increase the number of aberrations, whereas the commercial 2,4-D formulation (0.125, 0.250, 0.500, 1.000 and 1.250 mM, with respect to phenoxyacetic acid concentration) significantly increased the number of chromosome aberrations in vitro (without exogenous metabolic activation). The phenoxy acid levels in the breathing zone of the workers varied between 0.3 and 0.4 mg/m3, and the concentrations of phenoxyacetic acids in the urine of the workers after exposure varied from 0.000 to 0.055 mmol/l. There were no increases in chromosome aberrations in peripheral lymphocytes of the exposed subjects.

Cohen FL. 1986 Mar. Paternal contributions to birth defects. Nurs Clin North Am 21(1):49-64. Abstract: The question of the extent of paternal contributions to birth defects and adverse reproductive outcomes gained new urgency with the concerns of Vietnam veterans exposed to Agent Orange. There is well-established evidence for the occurrence of certain autosomal dominant sporadic mutations in the offspring of older fathers. Paternal nondisjunction at meiosis or mitosis accounts for a significant proportion of chromosomal errors such as trisomy 21. Certain chromosomal abnormalities in males also contribute to adverse reproductive outcomes in relation to repetitive spontaneous abortion and infertility. The question of whether or not teratogens can act through the male is complex. While certain suggestive evidence is reviewed here, conclusive data are yet to be found. Yet, we must remember that it was not long ago that the placenta was assumed to be an impenetrable barrier between mother and fetus. This field is ripe for further well-designed and careful investigation.

Blakley BR, Blakley PM. 1986 Feb. The effect of prenatal exposure to the n-butylester of 2,4-dichlorophenoxyacetic acid (2,4-D) on the immune response in mice. Teratology 33(1):15-20. Abstract: Pregnant CD-1 mice were administered the n-butylester of 2,4-dichlorophenoxyacetic acid (2,4-D) by gastric intubation on day 11 of gestation at dosages ranging from 0 to 200 mg/kg (2,4-D content). The immune response in the female offspring was elevated at 6 weeks of age. The humoral immune response, antibody production against sheep red blood cells, was not altered by 2,4-D ester exposure during gestation. The mitogen responses of lymphocytes induced by concanavalin A, a T-lymphocyte mitogen, or by Escherichia coli lipopolysaccharide, a B-lymphocyte mitogen, were reduced in the highest exposure group (200 mg/kg), although the T-lymphocyte suppression was not statistically significant. A similar response pattern was

observed in the background nonstimulated lymphocyte cultures, suggesting that the suppression was a generalized lymphocyte abnormality. Evaluation of the mitogen responses using stimulation indices to correct for the variable background responses demonstrated that 2,4-D produced no net suppressive effect in any of the treatment groups. Since in utero 2,4-D ester exposure produced no alterations in humoral immunity and only subtle effects on lymphocyte blastogenesis, it is unlikely to be of any immunotoxicological or immunoteratological significance. Further studies investigating commercial-grade 2,4-D formulations are necessary since these formulations contain other components that may potentially induce alterations in the immune system.

Hatch MC, Stein ZA. 1986. Agent Orange and risks to reproduction: the limits of epidemiology. Teratog Carcinog Mutagen 6(3):185-202.

[Anonymous]. 1986. Occupational exposures to chlorophenoxy herbicides. IARC Monogr Eval Carcinog Risk Chem Hum 41:357-406.

Mohammad FK, St Omer VE. 1985 Dec. Developing rat brain monoamine levels following in utero exposure to a mixture of 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acids. Toxicol Lett 29(2-3):215-23. Abstract: Pregnant rats were gavaged with a 1:1 mixture of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) at 0 (G0), 50 (G 50) and 100 (G 100) mg/kg per day on gestational days 6-15. Treatment significantly (P less than 0.05) delayed ontogeny of dopamine (DA), but not norepinephrine (NE) levels, in the thalamus-hypothalamus on postnatal day 7; in the pons-medulla on days 7,9 and 15; and in the olfactory lobes on day 9. On day 25, serotonin (5-HT) levels were significantly decreased in the pons-medulla of G 100 rats, whereas 5-hydroxyindoleacetic acid (5-HIAA) levels decreased in the thalamus-hypothalamus and pons-medulla of G 50 and G 100 rats.

Pearn JH. 1985 Nov. Herbicides and congenital malformations: a review for the paediatrician. Aust Paediatr J 21(4):237-42. Abstract: The herbicides 2, 4, 5-T and 2, 4-D are relatively non-toxic to primates, in acute exposure. Dioxins, which have occurred as impurities in these two herbicides, manifest universal biological toxicity. The best understood dioxin TCDD, has, in susceptible strains of mice, a very low teratogenic minimal effective dose of 1-10 micrograms/kg. This fact has engendered an era of uncertainty about the potential teratogenic effects of herbicides, in the context of potential human exposure. This paper reviews current knowledge concerning herbicide teratogenesis following maternal exposure. Because of species specificity of teratogenic agents, it is not possible to extrapolate from effects in lower animals to potential effects in humans. It remains a fact however that all proven human teratogens have parallel animal models. Following maternal exposure to herbicides and to dioxins, it has not been possible to produce teratogenic effects in primates, although fertility may be affected. Epidemiological reports from Hungary, Italy (the ICMESA accident), New Zealand, the United States, Europe and Australia have not revealed any positive evidence to indicate that a human herbicide teratogenic syndrome exists.

Preslan MW, Beauchamp GR, Zakov ZN. 1985 Sep-Oct. Congenital glaucoma and retinal dysplasia. J Pediatr Ophthalmol Strabismus 22(5):166-70. Abstract: The differential diagnosis of leukocoria (pseudoglioma) in the neonate includes multiple conditions, including malformations with retinal dysplasia as a component. Typically bilateral, retinal dysplasia is characteristically seen in microphthalmic eyes. Certain chromosomal defects have been described. The case reported herein presented in the first month of life with an enlarged eye, elevated intraocular pressure, prominent iris vasculature, and leukocoria. Family history was positive in one respect: this is the second child of a Viet Nam veteran exposed to Agent Orange. The first child, from a different mother, also had birth defects. Other than his left eye, the child is completely normal. Ultrasonography showed posterior vitreous opacities of indeterminate configuration. CT scan suggested a posterior intraocular mass. Histologically, the principal features were an anomalous, largely unformed corneoscleral angle, intraocular hemorrhage, and retinal dysplasia. Light microscopic studies were performed. The corneoscleral angle revealed an anteriorly inserted iris with an absence of trabecular meshwork and Schlemm's canal. This case is considered unique on the basis of the association of retinal dysplasia with congenital glaucoma and larger-than-normal eye. The significance of reported paternal exposure to Agent Orange in this instance is unknown.

Sterling TD, Arundel A. 1985 Aug 2. Vietnam veterans risk for fathering children with birth defects. JAMA

254(5):609-10.

Turkula TE, Jalal SM. 1985 May-Jun. Increased rates of sister chromatid exchanges induced by the herbicide 2,4-D. J Hered 76(3):213-4.Abstract: The potential for genetic damage from widely used hormonic herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D), continues to be of serious concern. The mutagenic effect as reflected by the rates of sister chromatid exchanges (SCE) was determined in cultured human lymphocytes. Data were based on the analysis of 50 cells for the control and each of the three treatments. A 50 micrograms/ml dosage caused a highly significant increase in SCE. Dosages of 100 and 250 micrograms/ml elevated the rate of SCE, but not significantly. Since 2,4-D biodegrades rapidly in soil and water, its continued use is not in serious question until safer compounds are available. However, the results of this study suggest that the danger of genetic damage from direct exposure to commercial samples of 2,4-D should not be ignored.

Kaye CI, Rao S, Simpson SJ, Rosenthal FS, Cohen MM. 1985. Evaluation of chromosomal damage in males exposed to agent orange and their families. J Craniofac Genet Dev Biol Suppl 1:259-65.Abstract: Agent Orange (AO), a phenoxyherbicide, and dioxin, an impurity found in AO, are considered clastogens, mutagens, and teratogens in plants and animals. AO has come under suspicion in humans following claims that it causes chromosome damage and birth defects in offspring of exposed individuals. No well-designed epidemiological studies are available to support this conclusion. Of ten exposed individuals studied for chromosome breaks and sister chromatid exchange frequencies, eight were ascertained because they had children with congenital defects. No consistent pattern of anomalies was observed. Five children had neurologic deficit, one child had a central nervous system anomaly, and one child was affected with glaucoma. Although all individuals studied had normal karyotypes, a statistically significant increase in chromosome breakage was observed in exposed males compared to their unexposed wives and children; sister chromatid exchange frequency was not increased.

Rashid KA, Babish JG, Mumma RO. 1984 Nov-Dec. Potential of 2,4-dichlorophenoxyacetic acid conjugates as promutagens in the Salmonella/microsome mutagenicity test. J Environ Sci Health B 19(8-9):689-701.Abstract: Hepatic S9 preparations from Aroclor 1254 induced rats and 3-methylcholanthrene induced woodchucks were used to investigate, in vitro, the mutagenic potential of five amino acid conjugates of 2,4-Dichlorophenoxyacetic acid (alanine, aspartic acid, leucine, methionine and tryptophan). Five strains of Salmonella typhimurium (TA97, TA98, TA100, TA1535, TA1538) were utilized for this purpose. Dose-response effects producing a two-fold increase of revertants over spontaneous levels were not observed with either S9 preparation indicating that the amino acid conjugates are not promutagens in these assays.

Erickson JD, Mulinare J, McClain PW, Fitch TG, James LM, McClearn AB, Adams MJ Jr. 1984 Aug 17. Vietnam veterans' risks for fathering babies with birth defects. JAMA 252(7):903-12.Abstract: Vietnam veterans' risks for fathering babies with major structural birth defects were assessed using a case-control study. Information regarding military service in Vietnam was obtained from interviews with mothers and fathers of babies in case and control groups and from review of military records. Vietnam veterans, in general, did not have an increased risk of fathering babies with defects (all types combined; relative risk estimate, 0.97). Vietnam veterans who had greater estimated opportunities for Agent Orange exposure did not seem to be at greater risk for fathering babies with all types of defects combined. However, for a few specific types of defects the estimated risks were higher for subgroups of Vietnam veterans that may have had a greater likelihood of exposure to Agent Orange. These seemingly higher risks could be chance events, the result of some experience in the Vietnam service of the father, or the result of some other unidentified risk factor.

Dan BB. 1984 Aug 17. Vietnam and birth defects. JAMA 252(7):936-7.

[Anonymous]. 1984 Aug 17. Vietnam veterans' risks for fathering babies with birth defects. MMWR Morb Mortal Wkly Rep 33(32):457-9.

Mortelmans K, Haworth S, Speck W, Zeiger E. 1984 Aug. Mutagenicity testing of agent orange components and related chemicals. Toxicol Appl Pharmacol 75(1):137-46.Abstract: Components of the herbicide Agent Orange--2,4-dichlorophenoxyacetic acid (2,4,-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and their esters, and the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)--and related chemicals were tested

for mutagenicity using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537. No mutagenic activity was observed for any of the chemicals tested.

Friedman JM. 1984 Apr. Does Agent Orange cause birth defects? Teratology 29(2):193-221. Abstract: Large quantities of the defoliant, Agent Orange, were sprayed in Vietnam during the war. Agent Orange was composed of two herbicides: 2,4-D and 2,4,5-T, the latter contaminated by small amounts of a highly toxic dioxin (TCDD). The constituents of Agent Orange are capable of producing gene mutations and chromosomal aberrations, at least in some experimental circumstances. TCDD and 2,4,5-T are teratogenic in mice and perhaps in other mammals, but the teratogenicity of these chemicals has not been convincingly demonstrated in humans. There is currently no scientific evidence which indicates that men who were previously exposed to Agent Orange are at increased risk of having children with birth defects, but available data are inadequate to assess this possibility critically.

Casey PH, Collie WR. 1984 Feb. Severe mental retardation and multiple congenital anomalies of uncertain cause after extreme parental exposure to 2,4-D. J Pediatr 104(2):313-5.

Linnainmaa K. 1984. The effects of hypolipidemic peroxisome proliferators on the induction of sister chromatid exchanges. Basic Life Sci 29 Pt B:965-74.

Fagan K, Pollak JK. 1984. The effect of the phenoxyacetic acid herbicides 2,4,5-trichlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid as ascertained by direct experimentation. Residue Rev 92:29-58.

Center for Disease Control, Center for Environmental Health, Chronic Diseases Div. 1984. Vietnam veterans' risks for fathering babies with birth defects. Morbidity & Mortality Weekly Report 33(32):457-459. Abstract: Vietnam veterans' risks for fathering babies born with serious structural brith defects were assessed using a case-control study.

Reuber MD. 1983 Dec 1. Carcinogenicity and toxicity of 2,4-dichlorophenoxy-acetic acid. Sci Total Environ 31(3):203-18. Abstract: 2,4-Dichlorophenoxyacetic acid (2,4-D) is carcinogenic in male and female rats and probably also in mice. Male and female rats ingesting 2,4-D developed increased incidences of malignant neoplasms. Lymphosarcomas were increased in rats of both sexes, and neoplasms of the mammary gland in female rats. Male rats also had carcinomas of the endocrine organs. 2,4-D isooctyl ester was carcinogenic for the lymphoreticular system in female mice. 2,4-D and 2,4-dichlorophenol also were promoters of neoplasms of the skin in mice. Male mice given 2,4-D isopropyl ester developed an increased incidence of neoplasms of the lung. 2,4-D also is mutagenic and teratogenic in animals and causes poisoning in animals and human beings.

[Anonymous]. 1983 Aug 25. Agent Orange and birth defects. N Engl J Med 309(8):491-2.

Gunby P. 1983 May 27. More questions, not answers, emerge from Agent Orange studies. JAMA 249(20):2743-6.

LaVecchio FA, Pashayan HM, Singer W. 1983 Mar 24. Agent Orange and birth defects. N Engl J Med 308(12):719-20.

Linnainmaa K. 1983. Sister chromatid exchanges among workers occupationally exposed to phenoxy acid herbicides 2,4-D and MCPA. Teratog Carcinog Mutagen 3(3):269-79. Abstract: The induction of sister chromatid exchanges (SCEs) was studied in the peripheral lymphocytes of workers spraying foliage in forestry with phenoxy acid herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) or their mixtures. In order to follow possible exposure-related changes in the frequencies of SCEs, three successive blood samples were taken from 50 male sprayers during the spraying season of July-October, 1981. In addition, 15 control subjects not working with herbicides were included in the study. The actual exposure levels of the exposed subjects were estimated by measuring the concentrations of 2,4-D and MCPA in the urine of the sprayers. Enough cells for the SCE analysis were obtained from 35 herbicide workers and 15 control subjects. The concentrations of 2,4-D and MCPA in the urine samples after exposure varied from 0.00 to 10.99 mg/l. No significant differences in the frequencies

of SCEs were observed in samples taken before, during, or after the exposure. Furthermore, the means of SCEs in a nonexposed control group of 15 subjects fell in the same range as those of the exposed subjects. A difference in the means of SCEs was observed between nonsmokers and smokers, smokers having significantly higher mean values than nonsmokers. The results of the present study add support to the earlier data indicating that 2,4-D and MCPA do not act as direct DNA-damaging agents.

Korte C, Jalal SM. 1982 May-Jun. 2,4-D induced clastogenicity and elevated rates of sister chromatid exchanges in cultured human lymphocytes. J Hered 73(3):224-6.Abstract: Potential for genetic damage in future generations from such widely used hormonic herbicide as 2,4-D (2,4-dichlorophenoxyacetic acid) is of serious concern. Yet the data, particularly on mammalian systems, continue to be inadequate and inconclusive. An attempt was made in this study to determine the clastogenic and mutagenic potential of 2,4-D in cultured lymphocytes. Chromosome damage though statistically insignificant occurred at dosages as low as 0.2 microgram/ml. Chromosome damage was increased at a statistically significant level whenever the concentration was 50 microgram/ml or higher. Mutagenicity, based on rates of increase in sister chromatid exchanges, was significant at 10 micrograms/ml of higher concentrations. Statistical testing was based o analysis of variance, Dunnett's multiple comparison tests and linear regressions. It seems imperative therefore to avoid indiscriminate use of 2,4-D, and to test the compound for long-range low-level exposures.

Lamb JC 4th, Moore JA, Marks TA, Haseman JK. 1981 Nov-Dec. Development and viability of offspring of male mice treated with chlorinated phenoxy acids and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Toxicol Environ Health 8(5-6):835-44.Abstract: Male C57BL/6 mice were given feed containing various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) such that their diets contained daily doses of approximately 40 mg/kg 2,4,-D, 40 mg/kg 2,4,5-T, and 2.4 micrograms/kg TCDD (group II); 40 mg/kg 2,4-D, 40 mg/kg 2,4,5-T, and 0.16 microgram/kg TCDD (group III); or 20 mg/kg 2,4-D, 20 mg/kg 2,4,5-T, and 1.2 micrograms/kg TCDD (group IV). Controls (group I) were given a diet with only the corn oil vehicle added to the feed. At the conclusion of an 8-wk dosing period, treated males cohabited with untreated virgin C57BL/6 female mice. Females were randomly assigned either to undergo teratological examination at 18 d of gestation, when the fetuses were examined for external, visceral, and skeletal malformations, or to be segregated and allowed to deliver their pups. Live and dead offspring and birth weight were recorded and viability was monitored until postnatal d 21. In both cases, development and survival of offspring in the test groups, whose sire had been treated with the mixture of phenoxy acids and TCDD, were not significantly different from those in the control group, whose sire had received untreated feed.

Duffard R, Traini L, de Duffard AM. 1981. Embryotoxic and teratogenic effects of phenoxy herbicides. Acta Physiol Lat Am 31(1):35-8.

Carver JH, Adair GM, Wandres DL. 1980 Sep. Mutagenicity testing in mammalian cells. II. Validation of multiple drug-resistance markers having practical application for screening potential mutagens. Mutat Res 72(2):207-30.Abstract: Chinese hamster ovary (CHO) cell lines heterozygous at both the adenine phosphoribosyltransferase (aprt) and thymidine kinase (tk) loci were used for single-step selection of spontaneous and induced mutants resistant to 8-azaadenine (AAr), 6-thioguanine (TGr), ouabain (OUAR), or 5-fluorodeoxyuridine (FUdRr). Mutation data are reported for direct mutagens (EMS, ethyl methanesulfonate; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; NQO, 4-nitroquinoline 1-oxide) and promutagens (DMN, dimethylnitrosamine; BP, benzo[a]-pyrene) activated by rat-liver homogenates. Optimal plating densities were established for AAr, TGr, OUAR and FUdRr. The induced mutant frequencies as a function of relative cell survival after treatment with EMS, DMN or BP were 2--4 d for AAr, 6--8 d for TGr, 3 d for OUAR, and 1--3 d for FUdRr. The induced mutant frequencies as a function of relative cell survival after treatment with EMS, DMN or BP showed locus-specific differences in sensitivity. Of 61 clonal isolates resistant to AA and assayed for APRT activity, 87% had less than or equal to 5% wild-type activity; of 30 TGr clones assayed, 83% had less than or equal to 5% wild-type HGPRT activity. Of 42 FUdRr clones assayed, 98% had less than or equal to 1% wild-type TK activity. 50 clones selected in medium containing FUdR displayed cross-resistance to 5-bromodeoxyuridine (BUdR) and trifluorothymidine (TFT) and all were sensitive to HAT (hypoxanthine--amethopterin--thymidine) medium. The tk locus showed the largest mutational response as a function of cell survival after mutagen treatment.

The rapid expression kinetics for FUdRr and the possibility that the locus detects a broader spectrum of genetic lesions than the other drug-resistance markers are discussed in terms of a sensitive screening assay for detecting potential mutagens.

Seiler JP. 1978. The genetic toxicology of phenoxy acids other than 2,4,5-T. Mutat Res 55(3-4):197-226.

Ahmed FE, Hart RW, Lewis NJ. 1977 Feb. Pesticide induced DNA damage and its repair in cultured human cells. Mutat Res 42(2):161-74. Abstract: The effects of pesticides on the induction of unscheduled DNA synthesis in SV-40 transformed human cells (VA-4) in culture with and without metabolic activation by liver microsomes was studied. Results showed that ten of the thirteen compounds examined either directly or upon metabolic activation induced unscheduled DNA synthesis in the human cell system used. The DNA repair kinetics and size of the repaired regions resulting from treatment with four of the chemicals (Carbaryl, Chlordane, Dieldrin and 2.4-D Fluid) were studied by 313 nm photolysis of repaired regions containing bromodeoxyuridine (BUdR). The size of the repaired regions differed between compounds but could generally be classified as either of the X-ray (short) or UV-type (long).

Courtney KD. 1977. Prenatal effects of herbicides: evaluation by the prenatal development index. Arch Environ Contam Toxicol 6(1):33-46. Abstract: The herbicides 2,4-D and 2,4,5-T and many of the esters of these compounds produced cleft palates in CD-1 mice. Silvex and Agent Orange also produced cleft palates. Depending on the dose and means of administration variable responses relating to the production of cleft palates, effect on fetal weight, and effect on fetal mortality were obtained. In order to view these data comprehensively, the Prenatal Development Index was determined. This index was computed from the incidence of malformed fetuses, fetal mortality and fetal body weight. This made it possible to evaluate data from some experiments with feto-toxic doses of compounds in which the high incidence of fetal mortality and consequently low incidence of viable fetuses obscured the response.

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