References

Behrens,M.R., N.Mutlu, S.Chakraborty, R.Dumitru, W.Z.Jiang, B.J.Lavallee, P.L.Herman, T.E.Clemente, and D.P.Weeks. 2007. "Dicamba resistance: enlarging and preserving biotechnology-based weed management strategies." *Science*. 316:1185-1188.
Abstract: The advent of biotechnology-derived, herbicide-resistant crops has revolutionized farming practices in many countries. Facile, highly effective, environmentally sound, and profitable weed control methods have been rapidly adopted by crop producers who value the benefits associated with biotechnology-derived weed management traits. But a rapid rise in the populations of several troublesome weeds that are tolerant or resistant to herbicides currently used in conjunction with herbicide-resistant crops may signify that the useful lifetime of these economically important weed management traits will be cut short. We describe the development of soybean and other broadleaf plant species resistant to dicamba, a widely used, inexpensive, and environmentally safe herbicide. The dicamba resistance technology will augment current herbicide resistance technologies and extend their effective lifetime. Attributes of both nuclear- and chloroplast-encoded dicamba resistance genes that affect the potency and expected durability of the herbicide resistance trait are examined

Caux,P.Y., R.A.Kent, M.Tache, C.Grande, G.T.Fan, and D.D.MacDonald. 1993. "Environmental fate and effects of dicamba: a Canadian perspective." *Rev.Environ.Contam Toxicol.* 133:1-58.
Abstract: Literature on the environmental fate and effects of the benzoic acid herbicide dicamba was reviewed to provide a scientific basis to derive Canadian Water Quality Guidelines. Included in the review was information on the uses and production of dicamba, its physical and chemical properties, environmental monitoring data in Canadian surface water and groundwater, soils, sediments, and biota, and its environmental degradation, persistence, and fate. Through monitoring, dicamba has been detected in less than 8% of surface-water samples to a maximum concentration of 13 micrograms.L-1, while 2% of groundwater samples were positive up to 517 micrograms.L-1. Only one study that analyzed sediments (with no detections) and no field studies that investigated residues in biota were found. Microbial degradation is the most important process governing the dissipation of dicamba in aquatic and soil environments. Photolysis, hydrolysis, volatilization, adsorption to sediment, and bioconcentration are not expected to be significant removal processes, based on limited environmental fate data. The half-life of dicamba in water is < 7 d, although residues have been detected in surface-water supplies in Alberta more than 6 mon after application. The literature reports the half-life in soils ranges from 4 to 555 d; however, < 12 wk would be typical under Canadian conditions. High moisture and temperature, and other conditions that favor microbial degradation, would likely reduce the half-life to < 4 wk. The principal soil and plant metabolite is 3,6-dichlorosalicylic acid, with minor amounts of 2,5-dihydroxy-3,6-dichlorobenzoic acid and 5-hydroxydicamba found. Dicamba is highly mobile in soil, and significant leaching is possible; its water solubility is 6.5 g.L-1 (25 degrees C) and it has a log octanol-water partition coefficient of 0.477. Acute and chronic toxicological studies for all nontarget plants and animals were also reviewed. The major groups of organisms for which toxicological data were collected were freshwater fish, invertebrates and plants, tame hays and cereals, legumes, and other crops, and livestock poultry and mammals. The acute toxicity (< or = 96-hr LC50) to freshwater fish ranged from 28 to 516 mg.L-1, whereas that for invertebrates ranged from 3.9 to > 100 mg.L-1. No chronic data were found for either of these groups. The chronic EC50 to 14 freshwater algae, based on growth inhibition, ranged from 100 to > 10,000 micrograms.L-1. No studies on freshwater macrophytes or any marine organisms were found. Agricultural crops exhibited varying toxicity.(ABSTRACT TRUNCATED AT 400 WORDS)

Cavieres,M.F., J.Jaeger, and W.Porter. 2002. "Developmental toxicity of a commercial herbicide mixture in mice: I. Effects on embryo implantation and litter size." *Environ.Health Perspect.* 110:1081-1085.
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

Donald,D.B., N.P.Gurprasad, L.Quinnett-Abbott, and K.Cash. 2001. "Diffuse geographic distribution of herbicides in northern prairie wetlands." *Environ.Toxicol.Chem.* 20:273-279.
Abstract: The concentrations of herbicides in water from wetlands on landscapes where herbicides are not used should be less than on farms with moderate (conventional farms) and intense (minimum-till farms) herbicide use. In general, this hypothesis was not supported for wetlands situated in the Boreal Plains Ecozone of central Saskatchewan, Canada. The overall detection frequency of 10 commonly used herbicides was not significantly different among wildlife habitat with no pesticide use (44.4%), farms with no pesticide use (51.6%), conventional farms (54.9%), and minimum-till farms (56.5%, chi 2 = 5.64, p = 0.13). The herbicides (4-chloro-2-methylphenoxy) acetic acid (MCPA), 2,4-dichlorophenoxyacetic acid (2,4-D), bromoxynil, dicamba, mecoprop, and diclorprop accounted for 87% of all detections. In general, detection frequencies and concentrations of individual herbicides were similar on all land-use types. For example, the mean concentration of 2,4-D in water on the four land types ranged from 0.12 +/- 0.104 to 0.26 +/- 0.465 microgram/L, and MCPA ranged from 0.08 +/- 0.078 to 0.19 +/- 0.166 microgram/L. However, in the year of application, mean concentrations of MCPA and bromoxynil, but not 2,4-D, were significantly higher by about twofold in wetlands situated in fields where these herbicides were applied compared with all other wetlands. We propose that many agricultural pesticides are rapidly lost to the atmosphere at the time of application by processes such as volatilization from soil and plant evapotranspiration. Then, the herbicides used throughout the region may be directly absorbed to the surface of wetlands from the atmosphere, or they become entrained in local convective clouds, and are redistributed by rainfall in a relatively homogenous mixture over the agricultural landscape. The low levels of individual herbicides we found in most of the wetland waters would not cause chronic effects to aquatic biota

Espandiari,P., H.P.Glauert, E.Y.Lee, and L.W.Robertson. 1999. "Promoting activity of the herbicide dicamba (2-methoxy-3, 6-dichlorobenzoic acid) in two stage hepatocarcinogenesis." *Int.J.Oncol.* 14:79-84.
Abstract: Our goal was to examine whether dicamba, a widely-used broad leaf herbicide, has promoting activity in two-stage hepatocarcinogenesis. Female Sprague Dawley rats were given a single dose of diethylnitrosamine and then diets containing dicamba, or phenobarbital, or both for six months. The number and volume of placental

glutathione-S-transferase-positive, glucose-6-phosphatase-negative or ATPase-negative foci were quantified in the liver. Dicamba alone did not increase the number or volume of these altered hepatic foci. Dicamba did, however, show a significant effect on the number or volume of certain markers in animals also treated with phenobarbital. These data show that dicamba in combination with other promoters may have weak promoting activity in two-stage hepatocarcinogenesis in the rat

Filkowski,J., J.Besplug, P.Burke, I.Kovalchuk, and O.Kovalchuk. 2003. "Genotoxicity of 2,4-D and dicamba revealed by transgenic Arabidopsis thaliana plants harboring recombination and point mutation markers." *Mutat.Res.* 542:23-32.
Abstract: The phenoxy herbicides 2,4-D and dicamba are released daily into the environment in large amount. The mechanisms of genotoxicity and mutagenicity of these herbicides are poorly understood, and the available genotoxicity data is controversial. There is a cogent need for a novel genotoxicity monitoring system that could provide both reliable information at the molecular level, and complement existing systems.We employed the transgenic Arabidopsis thaliana 'point mutation' and 'recombination' plants to monitor the genetic effects of the herbicides 2,4-D and dicamba. We found that both herbicides had a significant effect on the frequency of homologous recombination A-->G mutation. Neither herbicides affected the T-->G mutation frequency. Interestingly, these transgenic biomonitoring plants were able to detect the presence of phenoxy herbicides at concentrations that were lower than the guideline levels for Drinking Water Quality. The results of our studies suggest that our transgenic system may be ideal for the evaluation of the genotoxicity of herbicide-contaminated water. Moreover, the unique ability of the plants to detect both double-strand breaks (homologous recombination) and point mutations provides tremendous potential in the study of molecular mechanisms of genotoxicity and mutagenicity of phenoxy herbicides

Gibb,C., T.Satapanajaru, S.D.Comfort, and P.J.Shea. 2004. "Remediating dicamba-contaminated water with zerovalent iron." *Chemosphere*. 54:841-848.
Abstract: Dicamba (3,6-dichloro-2-methoxybenzoicacid) is a highly mobile pre- and post-emergence herbicide that has been detected in ground water. We determined the potential of zerovalent iron (Fe0) to remediate water contaminated with dicamba and its common biological degradation product, 3,6-dichlorosalicylic acid (DCSA). Mixing an aqueous solution of 100 microM dicamba with 1.5% Fe0 (w/v) resulted in 80% loss of dicamba within 12 h. Solvent extraction of the Fe0 revealed that dicamba removal was primarily through adsorption; however when the Fe0 was augmented with Al or Fe(III) salts, dicamba was dechlorinated to an unidentified degradation product. In contrast to dicamba, Fe0 treatment of DCSA resulted in removal with some dechlorination observed. When DCSA was treated with Fe0 plus Al or Fe(III) salts, destruction was 100%. Extracts of this Fe0 treatment contained the same HPLC degradation peak observed with the Fe0 + Al or Fe(III) salt treatment of dicamba. Molecular modeling suggests that differences in removal and dechlorination rates between dicamba and DCSA may be related to the type of coordination complex formed on the iron surface. Experiments with 14C-labeled dicamba confirmed that Fe-adsorbed dicamba residues are available for subsequent biological mineralization (11% after 125 d). These results indicate that Fe0 could be potentially used to treat dicamba and DCSA-contaminated water

Gonzalez,N.V., S.Soloneski, and M.L.Larramendy. 2006. "Genotoxicity analysis of the phenoxy herbicide dicamba in mammalian cells in vitro." *Toxicol.In Vitro*. 20:1481-1487.
Abstract: The cytogenetic effects exerted by the phenoxy herbicide dicamba and one of its commercial formulations banvel (57.71% dicamba) were studied in in vitro whole blood human lymphocyte cultures. The genotoxicity of herbicides was measured by analysis of the frequency of sister chromatid exchanges (SCEs) and cell-cycle progression assays. Both dicamba and banvel activities were tested within 10.0-500.0 microg/ml doses range. Only concentrations of 200.0 microg/ml of dicamba and 500.0

microg/ml of banvel induced a significant increase in SCE frequency over control values. The highest dose of dicamba tested (500.0 microg/ml) resulted in cell culture cytotoxicity. The cell-cycle kinetics was affected by both test compounds since a significant delay in cell-cycle progression and a significant reduction of the proliferative rate index were observed after the treatment with 100.0 and 200.0 microg/ml of dicamba and 200.0 and 500.0 microg/ml of banvel. For both chemicals, a progressive dose-related inhibition of the mitotic activity of cultures was observed. Moreover, only the mitotic activity statistically differed from control values when doses of both chemicals higher than 100.0 microg/ml were employed. On the basis of our results, the herbicide dicamba is a DNA damage agent and should be considered as a potentially hazardous compound to humans

Greenlee,A.R., T.M.Ellis, and R.L.Berg. 2004. "Low-dose agrochemicals and lawn-care pesticides induce developmental toxicity in murine preimplantation embryos." *Environ.Health Perspect.* 112:703-709.
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

Gu,J.G., C.Qiao, and J.D.Gu. 2003. "Biodegradation of the herbicides atrazine, cyanazine, and dicamba by methanogenic enrichment cultures from selective soils of China." *Bull.Environ.Contam Toxicol.* 71:924-932.

Hrelia,P., F.Vigagni, F.Maffei, M.Morotti, A.Colacci, P.Perocco, S.Grilli, and G.Cantelli-Forti. 1994. "Genetic safety evaluation of pesticides in different short-term tests." *Mutat.Res.* 321:219-228.
Abstract: Cyanazine, cyhexatin, dicamba and DNOC are pesticides commonly and broadly used in agriculture pest control. However, there is little information on their toxicity and mutagenicity in human cells and in whole animals. Therefore, UDS assay and SCE assay in human peripheral lymphocytes, and chromosome aberration analysis in bone marrow of rats have been used to assess the DNA-damaging activity of the above pesticides. Cyanazine proved non-genotoxic in all the test systems. Cyhexatin showed only weakly positive results for SCE induction in human lymphocytes, providing no concern for genotoxicological hazard. While dicamba did not show clastogenic effects in rodents, DNOC gave significant dose-related increases of structural chromosome aberrations in rat bone marrow cells. Female animals showed increased sensitivity to

the toxic effects by DNOC at the highest dose. The results provide further information on the intrinsic genotoxic activity of the tested pesticides, which may contribute to the toxicological assessment of the risk associated with human exposure

Kelley,K.B., K.N.Lambert, A.G.Hager, and D.E.Riechers. 2004. "Quantitative expression analysis of GH3, a gene induced by plant growth regulator herbicides in soybean." *J.Agric.Food Chem.* 52:474-478.
Abstract: Symptoms resembling off-target plant growth regulator (PGR) herbicide injury are frequently found in soybean fields, but the causal agent is often difficult to identify. The expression of GH3, an auxin-regulated soybean gene, was quantified from soybean leaves injured by PGR herbicides using real-time RT-PCR. Expression of GH3 was analyzed to ascertain its suitability for use in a diagnostic assay to determine whether PGR herbicides are the cause of injury. GH3 was highly induced by dicamba within 3 days after treatment (DAT) and remained high at 7 DAT, but induction was much lower at 17 DAT. GH3 was also highly induced at 7 DAT by dicamba + diflufenzopyr, and to a lesser extent by the other PGR herbicides clopyralid and 2,4-D. The non-PGR herbicides glyphosate, imazethapyr, and fomesafen did not significantly induce GH3 expression above a low constitutive level. These results indicate that a diagnostic assay for PGR herbicide injury based on overexpression of auxin-responsive genes is feasible, and that GH3 is a potential candidate from which a diagnostic assay could be developed. However, time course analysis of GH3 expression indicates the assay would be effective for a limited time after exposure to the herbicide

Kutz,F.W., B.T.Cook, O.D.Carter-Pokras, D.Brody, and R.S.Murphy. 1992. "Selected pesticide residues and metabolites in urine from a survey of the U.S. general population." *J.Toxicol.Environ.Health*. 37:277-291.
Abstract: Residues of toxic chemicals in human tissues and fluids can be important indicators of exposure. Urine collected from a subsample of the second National Health and Nutrition Examination Survey was analyzed for organochlorine, organophosphorus, and chlorophenoxy pesticides or their metabolites. Urine concentration was also measured. The most frequently occurring residue in urine was pentachlorophenol (PCP), found in quantifiable concentrations in 71.6% of the general population with an estimated geometric mean level of 6.3 ng/ml. Percent quantifiable levels of PCP were found to be highest among males. Quantifiable concentrations of 3,5,6-trichloro-2-pyridinol (5.8%), 2,4,5-trichlorophenol (3.4%), para-nitrophenol (2.4%), dicamba (1.4%), malathion dicarboxylic acid (0.5%), malathion alpha-monocarboxylic acid (1.1%), and 2,4-D (0.3%) were found, but at much lower frequencies. No quantifiable levels of 2,4,5-T or silvex were found. Preliminary analyses showed an apparent relationship between residue concentration and two measures of urine concentration (osmolality and creatinine). A large segment of the general population of the United States experienced exposure to certain pesticides, including some considered biodegradable, during the years 1976-1980

McDuffie,H.H., P.Pahwa, J.R.McLaughlin, J.J.Spinelli, S.Fincham, J.A.Dosman, D.Robson, L.F.Skinnider, and N.W.Choi. 2001. "Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health." *Cancer Epidemiol.Biomarkers Prev.* 10:1155-1163.
Abstract: Our objective in the study was to investigate the putative associations of specific pesticides with non-Hodgkin's Lymphoma [NHL; International Classification of Diseases, version 9 (ICD-9) 200, 202]. We conducted a Canadian multicenter population-based incident, case (n = 517)-control (n = 1506) study among men in a diversity of occupations using an initial postal questionnaire followed by a telephone interview for those reporting pesticide exposure of 10 h/year or more, and a 15% random sample of the remainder. Adjusted odds ratios (ORs) were computed using conditional logistic regression stratified by the matching variables of age and province of residence, and subsequently adjusted for statistically significant medical variables (history of

measles, mumps, cancer, allergy desensitization treatment, and a positive history of cancer in first-degree relatives). We found that among major chemical classes of herbicides, the risk of NHL was statistically significantly increased by exposure to phenoxyherbicides [OR, 1.38; 95% confidence interval (CI), 1.06-1.81] and to dicamba (OR, 1.88; 95% CI, 1.32-2.68). Exposure to carbamate (OR, 1.92; 95% CI, 1.22-3.04) and to organophosphorus insecticides (OR, 1.73; 95% CI, 1.27-2.36), amide fungicides, and the fumigant carbon tetrachloride (OR, 2.42; 95% CI, 1.19-5.14) statistically significantly increased risk. Among individual compounds, in multivariate analyses, the risk of NHL was statistically significantly increased by exposure to the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D; OR, 1.32; 95% CI, 1.01-1.73), mecoprop (OR, 2.33; 95% CI, 1.58-3.44), and dicamba (OR, 1.68; 95% CI, 1.00-2.81); to the insecticides malathion (OR, 1.83; 95% CI, 1.31-2.55), 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT), carbaryl (OR, 2.11; 95% CI, 1.21-3.69), aldrin, and lindane; and to the fungicides captan and sulfur compounds. In additional multivariate models, which included exposure to other major chemical classes or individual pesticides, personal antecedent cancer, a history of cancer among first-degree relatives, and exposure to mixtures containing dicamba (OR, 1.96; 95% CI, 1.40-2.75) or to mecoprop (OR, 2.22; 95% CI, 1.49-3.29) and to aldrin (OR, 3.42; 95% CI, 1.18-9.95) were significant independent predictors of an increased risk for NHL, whereas a personal history of measles and of allergy desensitization treatments lowered the risk. We concluded that NHL was associated with specific pesticides after adjustment for other independent predictors

Perocco,P., G.Ancora, P.Rani, A.M.Valenti, M.Mazzullo, A.Colacci, and S.Grilli. 1990. "Evaluation of genotoxic effects of the herbicide dicamba using in vivo and in vitro test systems." *Environ.Mol.Mutagen.* 15:131-135.
Abstract: The genotoxic effects of the herbicide dicamba have been studied by measuring 1) the unwinding rate of liver DNA from intraperitoneally (i.p.) treated rats (fluorimetric assay); 2) DNA repair as unscheduled DNA synthesis (UDS) induced in cultured human peripheral blood lymphocytes (HPBL); and 3) sister chromatid exchanges (SCE) in HPBL. Results show that dicamba is capable of inducing DNA damage since it significantly increases the unwinding rate of rat liver DNA in vivo and also induces UDS in HPBL in vitro in the presence of exogenous metabolic activation (S-9 mix). Furthermore, dicamba causes a very slight increase in SCE frequency in HPBL in vitro

Samanic,C., J.Rusiecki, M.Dosemeci, L.Hou, J.A.Hoppin, D.P.Sandler, J.Lubin, A.Blair, and M.C.Alavanja. 2006. "Cancer incidence among pesticide applicators exposed to dicamba in the agricultural health study." *Environ.Health Perspect.* 114:1521-1526.
Abstract: BACKGROUND: Dicamba is an herbicide commonly applied to crops in the United States and abroad. We evaluated cancer incidence among pesticide applicators exposed to dicamba in the Agricultural Health Study, a prospective cohort of licensed pesticide applicators in North Carolina and Iowa. METHODS: Detailed pesticide exposure information was obtained through a self-administered questionnaire completed from 1993 to 1997. Cancer incidence was followed through 31 December 2002 by linkage to state cancer registries. We used Poisson regression to estimate rate ratios and 95% confidence intervals for cancer subtypes by tertiles of dicamba exposure. Two dicamba exposure metrics were used: lifetime exposure days and intensity-weighted lifetime exposure days (lifetime days x intensity score). RESULTS: A total of 41,969 applicators were included in the analysis, and 22,036 (52.5%) reported ever using dicamba. Exposure was not associated with overall cancer incidence nor were there strong associations with any specific type of cancer. When the reference group comprised low-exposed applicators, we observed a positive trend in risk between lifetime exposure days and lung cancer ($p = 0.02$), but none of the individual point estimates was significantly elevated. We also observed significant trends of increasing risk for colon cancer for both lifetime exposure days and intensity-weighted lifetime days, although these results are largely due to elevated risk at the highest exposure level.

There was no apparent risk for non-Hodgkin lymphoma. CONCLUSIONS: Although associations between exposure and lung and colon cancer were observed, we did not find clear evidence for an association between dicamba exposure and cancer risk