2,4-Dichlorophenoxyacetic Acid (2,4-D) and Its Salts and Esters

2,4-D and its salts and esters are commonly used herbicides and plant-growth regulators. Registered uses include application to field, fruit, and vegetable crops including corn, wheat, barley, oats, sugar cane, rice, citrus, stone fruits, grapes, nuts and asparagus. 2,4-D and its salts and esters are also used on turf, lawns, rights-of-way, aquatic sites and forestry applications; as a plant growth regulator (predominately the isooctyl ester) applied pre-harvest in citrus to extend the shelf life (*e.g*., lemons) or the time fruit will remain in the tree without dropping (*e.g*., navel oranges). 2,4-D and its salts and esters are common ingredients in weed-and-feed fertilizers marketed to the general public for use on home lawns by residents. In 2009, a total of 1,233,338 pounds of 2,4-D and various of its salts and esters were sold in California, of which 10,594 pounds of 2,4-D and 485,000 pounds of various salts and esters were used for agricultural purposes. These substances have also been detected in drinking water and food. Exposure to 2,4-D may occur through the use of consumer products containing 2,4-D; during the handling, mixing, or application of the herbicides; by ingesting contaminated water and food; and through post-application exposure contact with treated areas (*e.g*., lawns, golf courses), including bodies of water treated with 2,4-D for aquatic weed control.

2,4-D and its salts and esters passed the human and animal data screens, underwent a preliminary toxicological evaluation, and are being brought to the Carcinogen Identification Committee (CIC) for consultation. This is a compilation of the relevant studies identified during the preliminary toxicological evaluation.

Epidemiological data

Human

- The epidemiologic evidence of carcinogenicity of chlorophenoxy herbicides was reviewed in 1987 by IARC who concluded there was limited evidence of 2,4-D human carcinogenicity for chlorophenoxy herbicides, based on findings of soft tissue sarcoma or non-Hodgkin's lymphoma with occupational exposure.

- The evidence was most recently reviewed by the Washington State Department of Ecology in 2001 who found the chlorophenoxy herbicide epidemiological studies presented "conflicting results and conclusions regarding an association between 2,4-D exposure and the development of non-Hodgkin's lymphoma (NHL) and/or soft tissue sarcoma (STS). However, a review of the literature specifically concerning exposure to 2,4-D appears to indicate that if there is an association, it is with NHL than STS."

Since 2001, several human studies have been published. Results are outlined below.

- Case-control studies of non-Hodgkin's lymphoma (NHL)
    - Population-based, in Canada: McDuffie *et al*. (2001)
        - Odds Ratio (OR) = 1.32 (95% CI 1.01 - 1.73)
    - Nested within a cohort of California farm workers: Mills *et al*. (2005)

        - OR = 3.80 (95% CI 1.85 – 7.81)
    - Population-based, in Italy: Miligi *et al.* (2006)
        - OR = 0.9 (95% CI 0.5-1.8) overall
        - OR = 4.4 (95% CI 1.1 - 29.1) for medium to high exposure and no protective equipment.

- Case-control studies of breast cancer
    - Nested within a cohort of California farm workers: Mills and Yang (2005)
        - 1988-1994: OR = 0.62 (95% CI 0.23 - 1.69) for high exposure.
        - 1995-2001: OR = 2.14 (95% CI 1.06 - 4.32) for high exposure.

- Case-control studies of stomach cancer
    - Nested within a cohort of California farm workers: Mills and Yang (2007)
      OR = 1.85 (95% CI 1.05 – 3.25)

- Cohort studies
    - Male employees involved in the manufacture or formulation of 2,4-D, from 1945 to 1994: Burns *et al.* (2001)
        - *No increased risk of any cancers associated with 2,4-D exposure*
    - Agricultural health cohort study on pesticide exposure and cancer incidence in Canada: Weichenthal *et al.* (2010)
        - *No increased risk of any cancers associated with 2,4-D exposure*

Canine

- Case-control study
    - Hospital-based case-control study of malignant lymphoma in pet dogs: Hayes *et al.* (1991)
        - *Increased risk of canine malignant lymphoma associated with application of 2,4-D to the home lawn 4 or more times per year*
        - Critique of original study published by Carlo *et al.* (1992)
        - Further analysis of original study data by Hayes *et al.* (1995)
        - Re-analysis of the original study data using different exposure definitions by Kaneene and Miller (1999)

Animal carcinogenicity data

2,4-D

- Long-term feeding studies
    - Two-year studies in male and female Osborne-Mendel rats: Hansen *et al.* (1971), as reviewed by Reuber (1983, pp. 204-210); IARC (1977)
        - *Increase in lymphosarcomas (by pairwise comparison and trend) and mammary gland tumors (by trend) in females*
        - *Increase in lymphosarcomas (by pairwise comparison and trend) and occurrence of rare subcutaneous neurosarcomas in males*

- 104-week studies (doses: 0, 1, 5, 15, 45 mg/kg/day) in male and female Fisher 344 rats: U.S. EPA (1997, pp. 6-7); CDPR (2006, p. 4).
  - *Increase in brain astrocytomas (by trend) in males*
  - *No treatment-related tumor findings in females*
- 104-week studies (doses: 0, 5, 75, and 150 mg/kg/day) in male and female Fisher 344 rats: U.S. EPA (1997, pp. 13-15); CDPR (2006, pp. 3-5)
  - *One brain astrocytoma was observed in high dose treated males*
  - *No treatment-related tumor findings in females*
- Two-year studies (male doses: 0, 5, 62.5, 125 mg/kg/day; female doses: 0, 5, 150, 300 mg/kg/day) in male and female $B6C3F_1$ mice: Charles *et al*. (1996, see Table 4); U.S. EPA (1997, pp. 11-13); CDPR (2006, pp. 8-9)
  - *Non-statistically significant increase in hepatocellular adenoma in females*
  - *No treatment-related tumor findings in males*

- Long-term drinking water study
  - One-year study in male Swiss (CD-1) mice: Blakley *et al*. (1992)
    - *No treatment-related increase in lymphocytic leukemia of thymic origin*

- Long-term gavage/diet studies
  - 2,4-D was given by gavage on days 7-28, then via diet until 78 weeks of age to male and female C57BL/6xC3H/AnF and C57BL/6xAKR mice: Innes *et al*. (1969), as reviewed by Reuber (1983, p. 21); IARC (1977)
    - *No treatment-related tumor findings were observed in males or females of either strain*

Individual salts and esters

2,4-D isooctyl ester

- Single subcutaneous (s.c.) injection studies
  - 78-week studies in male and female C57BL/6xC3H/AnF and C57BL/6xAKR mice: Reuber (1983, p. 213); IARC (1977)
    - *Increase in reticulum cell sarcomas (by pairwise comparison) in C57BL/6xAKR females*
    - *No treatment-related tumor findings in C57BL/6xC3H/AnF females or males of either strain*

- *Long-term* gavage/diet studies
  - 2,4-D isoocyl ester was given by gavage on days 7-28, then via diet until 78 weeks of age to male and female C57BL/6xC3H/AnF and

C57BL/6xAKR mice: Innes *et al*. (1969), as reviewed by Reuber (1983, pp. 212-213); IARC (1977)
    - *Slight increase in liver neoplasms in C57BL/6xC3H/AnF males* (numbers of animals with tumor not provided)
    - *Slight increase in lung tumors in C57BL/6xC3H/AnF females* (numbers of animals with tumor not provided)
    - *No treatment-related tumor findings in C57BL/6xAKR males or females*

2,4-D isopropyl ester

- Long-term gavage/diet studies
    - 2,4-D isopropyl ester was given by gavage on days 7-28, then via diet until 78 weeks of age to male and female C57BL/6xC3H/AnF and C57BL/6xAKR mice: Innes *et al*. (1969), as reviewed by Reuber (1983, pp. 211-212); IARC (1977)
        - *Marginal statistically significant (p = 0.0584) increase in neoplasms of lung in C57BL/6xC3H/AnF* males
        - *No treatment-related tumor findings in C57BL/6xAKR males or females of either strain*

2,4-D butyl ester

- Long-term gavage/diet studies
    - 2,4-D butyl ester was given by gavage on days 7-28, then via diet until 78 weeks of age to male and female C57BL/6xC3H/AnF and C57BL/6xAKR mice: Innes *et al*. (1969), as reviewed by Reuber (1983, p. 212); IARC (1977)
        - *Non-statistically significant increase in reticulum cell sarcoma in C57BL/6xAKR males*
        - *Non-statistically significant increase in reticulum cell sarcoma in C57BL/6xC3H/AnF females*
        - *No treatment-related tumor findings in C57BL/6xC3H/AnF males or C57BL/6xAKR females*

2,4-D amine salt

- Long-term feeding study
    - 27-month study in random-bred rats: Arkhipov and Koslova (1974), as reviewed by IARC (1977, p. 118)
        - *Occurrence of one mammary fibroadenoma and one hemangioma of the mesenterium*

Other relevant data

- 2,4-D Genotoxicity

  In vivo
    - Dominant lethal mutations

        - Mice *(negative*): IARC (1977)
    - Micronucleus formation
        - Human lymphocytes *(positive)*: Holland *et al*. (2002)
        - Mouse bone marrow *(negative):* U.S. EPA (1997); IARC (1977)
    - Sister chromatid exchange
        - Somatic and germ cells of mice *(positive)*: Madrigal-Bujaidar *et al*. (2001)
        - Chicken embryos *(positive)*: Arias (2003)
        - Human lymphocytes *(negative)*: U.S. EPA (1997); IARC (1987)
    - Chromosomal aberrations
        - Mouse bone marrow and spermatocytes (Swiss) *(positive)*: Amer and Aly (2001)
        - Rat bone marrow (Charles River, Wistar) *(equivocal)*: U.S. EPA (1997)
        - Human lymphocytes *(negative*): U.S. EPA (1997); IARC (1987)

In vitro

- Mutation assays: as reviewed in U.S. EPA (1997, pp. 16-19); IARC (1977, pp. 125-126)
    - *Salmonella* reverse mutation assays *(negative)*
    - *E. coli* mutation assays in strains *K12, WP2* and *PQ37(negative)*
    - *Drosophila melanogaster* sex-linked recessive lethal mutation assays in larvae *(positive);* in adults *(negative)*
    - Hamster hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) mutation assays in V79 fibroblasts *(positive)*
- Micronucleus formation
    - Human lymphocytes *(positive)*: Zeljezic and Garaj-Vrhovac (2004); Holland *et al*. (2002)
- Sister chromatid exchange
    - Human lymphocytes in presence of erythrocytes (2,4-D and its dimethylamine salt) *(positive)*: Soloneski *et al*. (2007)
    - Chinese hamster ovary (CHO) cells (2,4-D and its dimethylamine salt) *(positive)*: Gonzalez *et al*. (2005)
    - CHO cells *(positive without S9):* U.S. EPA (1997)
    - Rat lymphocytes *(negative)*: U.S. EPA (1997)
- Chromosomal aberration assays
    - Human lymphocytes *(positive)*: Zeljezic and Garaj-Vrhovac (2004);
    - Human lymphocytes *(positive and negative):* U.S. EPA (1997); IARC (1977)
    - CHO cells and embryonic bovine kidney and peripheral lymphocytes *(negative*): U.S. EPA (1997); IARC (1977)
- DNA strand breaks detected in the comet assay
    - CHO cells (2,4-D and its dimethylamine salt) *(positive)*: Gonzalez *et al.* (2005)
- DNA damage
    - Human fibroblasts *(negative)*: U.S. EPA (1997)

    - Unscheduled DNA synthesis
        - Fischer 344 rat primary hepatocytes *(negative):* U.S. EPA (1997)

- Mechanistic considerations
    - Endocrine disruption
        - 2,4-D alters thyroid hormone levels
            - Decreased serum T3 and T4 levels in male and female Wister rats: Kobal *et al.* (2000);
            - Decreased serum T4 in ewes: Rawlings *et al.* (1998)
        - Synergistic androgenic effect of 2,4-D and its metabolite 2,4-dichlorophenol in human prostate cancer cells *in vitro*: Kim *et al*. (2005)
    - Oxidative stress
        - 2,4-D increases lipid peroxidation (malondialdehyde = MDA) in rat liver, kidney and heart tissue *in vivo*: Celik *et al*. (2006)
        - 2,4-D increases lipid peroxidation (MDA) in male Wistar rat erythrocytes *in vivo*: Nakbi *et al.* (2010)
        - 2,4-D increases lipid peroxidation and hemolysis in human erythrocytes *in vitro*: Duchnowicz and Koter (2003)

Reviews

- IARC (1977; 1987)
- CDPR (2006)
- U.S. EPA (1997)
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