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2,4-Dichlorophenoxyacetic Acid (2,4-D) in Drinking Water

Guideline Technical Document for Public Consultation

Consultation period ends November 6, 2020

Canada

Purpose of consultation

This guideline technical document outlines the evaluation of the available information on 2,4-dichlorophenoxyacetic acid (2,4-D) with the intent of updating the guideline value for 2,4-D in drinking water. The purpose of this consultation is to solicit comments on the proposed guideline, on the approach used for its development, and on the potential economic costs of implementing them.

The existing guideline on 2,4-D, developed in 1991, based its maximum acceptable concentration of 0.1 mg/L (100 µg/L) on kidney toxicity in rats. This document proposes to reaffirm the maximum acceptable concentration (MAC) of 0.10 mg/L (100 µg/L) for 2,4-D in drinking water, based on kidney toxicity in rats.

This document is available for a 90-day public consultation period.
Please send comments (with rationale, where required) to Health Canada via email:

HC.water-eau.SC@canada.ca

If this is not feasible, comments may be sent by postal mail to:

Water and Air Quality Bureau, Health Canada
269 Laurier Avenue West, A.L. 4903D
Ottawa, ON K1A 0K9

All comments must be received before November 6, 2020. Comments received as part of this consultation will be shared with members of the Federal-Provincial-Territorial Committee on Drinking Water (CDW), along with the name and affiliation of their author. Authors who do not want their name and affiliation shared with CDW members should provide a statement to this effect along with their comments.

It should be noted that this guideline technical document will be revised following the evaluation of comments received, and a drinking water guideline will be established, if required. This document should be considered as a draft for comment only.

Proposed guideline

A maximum acceptable concentration (MAC) of 0.10 mg/L (100 µg/L) is proposed for 2,4-dichlorophenoxyacetic acid (2,4-D) in drinking water.

Executive summary

This guideline technical document was prepared in collaboration with the Federal-Provincial-Territorial Committee on Drinking Water and is based on assessments of 2,4-D completed by Health Canada's Pest Management Regulatory Agency (PMRA) and supporting documents.

Exposure

2,4-D is an herbicide used mainly to control broadleaf weeds. In 2016 (the most recent year for which data are available), it was one of the top 10 active ingredients sold in Canada. It is used on turf, forests, woodlots, terrestrial feed, food crops, and industrial and domestic non-food sites. Various forms of 2,4-D, including the free acid, salts and esters, are used in herbicide formulations and all release the acid as the active ingredient.

Exposure of Canadians to 2,4-D is expected to be low despite 2,4-D's widespread use. Low levels of 2,4-D in sources of drinking water have been found in many Canadian provinces. 2,4-D does not tend to accumulate in food, and inhalation exposure is not expected to be significant.

Health effects

Animal studies have consistently found 2,4-D affects the kidneys of mice and rats. There are no studies regarding kidney effects of 2,4-D in humans. Although some agencies consider 2,4-D to be possibly carcinogenic, international drinking water agencies have all assessed 2,4-D based on its non-cancer effects.

Analytical and treatment

The establishment of a drinking water guideline takes into consideration the ability to both measure the contaminant and remove it from drinking water supplies. 2,4-D can be detected at levels well below the proposed MAC of 0.10 mg/L.

Treatment technologies are available to effectively reduce 2,4-D concentrations in drinking water. Activated carbon adsorption is recognized as the best available technology. Biological filtration processes can also reduce 2,4-D concentrations. However, conventional treatment is not effective for 2,4-D removal. Typical oxidation/disinfection processes used in drinking water treatment also have limited potential to reduce 2,4-D concentrations.

At the residential scale, a number of certified treatment devices are currently available for the removal of 2,4-D. These devices rely mainly on adsorption (activated carbon) and reverse osmosis technologies.

Application of the guideline

Note: Specific guidance related to the implementation of drinking water guidelines should be obtained from the appropriate drinking water authority in the affected jurisdiction.

The proposed guideline for 2,4-D is protective against health effects from exposure to 2,4-D in drinking water over a lifetime. Any exceedance of the proposed MAC should be investigated and followed by the appropriate corrective actions if required. For exceedances in source water where there is no treatment in place, additional monitoring to confirm the exceedance should be conducted. If it is confirmed that source water 2,4-D concentrations are above the proposed MAC then an investigation to determine the most appropriate way to reduce exposure to 2,4-D should be conducted. This may include use of an alternate water supply or installation of treatment. Where treatment is already in place and an exceedance occurs, an investigation should be conducted to verify treatment and determine if adjustments are needed to lower the treated water concentration below the proposed MAC.

2,4-D is a chlorophenoxyacetic acid herbicide registered for commercial and domestic use in Canada to control broadleaf weeds. Applications can be made to agricultural crops, forested areas, lawn and turf (including residential uses), and other industrial sites. It is foliar-applied when weeds are actively growing, which, considering the broad use pattern, can be season-long (e.g., spring to fall). In areas of high use, 2,4-D can be introduced into surface water and possibly into groundwater through runoff and infiltration or as the result of spills. 2,4-D is non- to slightly-persistent in water and soil and undergoes rapid biological degradation under aerobic conditions. However, in oxygen-deprived environments such as anaerobic groundwater, the biological degradation of 2,4-D is rather limited.

International considerations

Other national and international organizations have drinking water guidelines, standards and/or guidance values. Variations in these values can be attributed to the age of the assessments or to differing policies and approaches, including the choice of key study and the use of different consumption rates, body weights and source allocation factors.

For 2,4-D, the U.S. Environmental Protection Agency has established a maximum contaminant level of 0.07 mg/L; the World Health Organization and the Australian National Health and Medical Research Council have established guideline values of 0.03 mg/L.

The European Union (EU) does not have a specific parametric value for individual pesticides. Instead, the EU has a value of 0.1 µg/L for any individual (single) pesticide, and a value of 0.5 µg/L for total pesticides found in drinking water. In establishing these values, the EU did not consider the science related to each pesticide, including health effects. Instead, the values are based on a policy decision to keep pesticides out of drinking water.

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1.0 Exposure Considerations

1.1 Sources and uses

As a selective systemic herbicide and indoleacetic acid (plant hormone) mimic, 2,4-dichlorophenoxyacetic acid or 2,4-D is mainly used to control broadleaf weeds (Charles et al., 1996a; WHO, 2003; US EPA, 2005; Health Canada, 2007). 2,4-D was on Health Canada's Pest Management Regulatory Agency's (PMRA) yearly list of "Top 10 Active Ingredients Sold in Canada" in 2016 (the most recent year for which data are available) with more than 1,000,000 kg of active ingredient (2,4-D) being sold for use on turf, forests, woodlots, terrestrial feed, food crops, and industrial and domestic non-food sites (Health Canada, 2016a, 2016b). It is foliar-applied when weeds are actively growing, which, considering the broad use pattern, can be season-long (e.g., spring to fall) (Health Canada, 2019).

Because of its low dissociation constant (2.8), 2,4-D will be present in its ionic form under the pH conditions typical of most Canadian soils and water bodies (pH 4.5–8.5). Since its amine salts dissociate in water within a few minutes to the acid anion and a conjugate cation, the environmental behaviour of the amine salts is comparable to that of 2,4-D. 2,4-D and its derivatives are non- to slightly persistent in soil and water. The main transformation products of 2,4-D are carbon dioxide, chlorohydroquinone and 2,4-dichlorophenol (2,4-DCP) as a minor fraction (Health Canada, 2007). 2,4-DCP is generally biodegradable, highly volatile and non-persistent in aerobic environments (Health Canada, 2007). 2,4-D is short lived in aerobic aquatic environments, with a half-life of less than 2 weeks, depending on water temperature, organic matter, bacterial composition and pH. The laboratory-derived aerobic biotransformation half-life of 2,4-D is 0.22–31 days in soil and 0.25–29 days in water (Health Canada, 2007).

Because of its high water solubility and low organic carbon adsorption coefficient, 2,4-D is expected to readily leach into groundwater if the downward flow of water is rapid; its soil mobility will increase with increasing pH and decreasing organic content (Johnson et al., 1995; Prado et al., 2001; US EPA, 2005; Health Canada, 2005a, 2007; HSDB, 2015). If 2,4-D's rate of movement through soil is slow, leaching will be offset by rapid biotransformation in the upper soil horizons and little 2,4-D will be found at depth, owing to its relatively short half-life in soil (Health Canada, 2007).

Under anaerobic conditions, biotransformation of 2,4-D is not significant and 2,4-D will persist in soil and aquatic environments. Anaerobic bacterial degradation in groundwater is relatively slow (half-life of 41–1610 days) (Health Canada, 2007).

Bioaccumulation is unlikely based on its low octanol-water partition coefficient (K_{ow}) and rapid degradation (WHO, 2003; Health Canada, 2007). Spray applications can lead to volatization, especially of the ester forms (Health Canada, 2007).

1.2 Substance identity

2,4-D (CAS Registry No. 94-75-7) is a white crystalline solid with a molecular formula of $C_8H_6Cl_2O_3$ and a molecular weight of 221.0 g/mol (US EPA, 2005; Health Canada, 2016b). Based on its physicochemical properties (see Table 1), it is very soluble in water, has a low potential to volatize and rapidly dissociates to its anionic form at environmental pHs (Health Canada, 2005a, 2007). Commercial products contain 2,4-D in a number of different forms: as free acid, as ester (butoxyethyl ester, 2-ethylhexyl ester), as amine (dimethylamine [DMA], isopropylamine [IPA], triisopropanolamine [TIPA]) and as choline salts (Health Canada, 2016b). The parent acid (2,4-D) is the herbicidally active portion, while the amine or ester portion allows for greater absorption into the plant (Health Canada, 2005a). The ester and amine forms dissociate quickly (<3 min) to the acid form both in the environment and within biological systems (Health Canada, 2007). Hence, when 2,4-D is mentioned in this document, it refers to the acid form. When the other forms are mentioned, their amounts will be expressed as 2,4-D. As 2,4-D's diethanolamine (DEA) and sodium salts are not transformation products of 2,4-D and their production and use have been discontinued in Canada (Health Canada, 2006), they will not be considered in this document.

2,4-D is often mixed with other herbicides and with additives such as antifoaming agents (Health Canada, 2005a; Kennepohl et al., 2010). In the past, manufacturing processes led to the presence of dioxins (e.g., 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,7,8-tetrachlorodibenzofuran) in 2,4-D preparations; however, with the implementation of new regulatory standards and improved production methods, dioxin levels are similar to or even lower than background levels in the environment (Health Canada, 2005a, 2006; Kennepohl et al., 2010).

Table 1. Properties of 2,4-D and some of its soluble salts relevant to its presence in drinking water.

	2,4-D	**Soluble salts of 2,4-D (DMA, IPA, TIPA)**	**Interpretation**[a]
CAS#	94-75-7[b]	2008-39-1, 5742-17-6, 32341-80-3[b]	
Molecular formula	$C_8H_6Cl_2O_3$	$C_{10}H_{13}Cl_2NO_3$, $C_{11}H_{15}Cl_2NO_3$, $C_{17},H_{27}Cl_2NO_6$ [b]	
Molecular weight	221.0 g/mol [b]	266.13, 280.04, 412.31[b]	
n-Octanol/water partition coefficient (log K_{ow})	0.04–2.14[a] at pH 5 and 25°C	Not available—salts dissociate to acid in water [b]	Unlikely to bioaccumulate
Henry's law constant	7.26×10^{-6} Pa m^3 mol^{-1}[a]	Not available—based on DMA salts dissociate rapidly to acid[d]	Non-volatile from water or moist surfaces
Specific gravity	1.416 at 25°C[b]	1.15–1.23 at 20°C [b]	
Water solubility	24.3 g/L[f] at 20°C	17.4–72.9 g/100 ml[d]	Very soluble in water
Organic carbon adsorption coefficient (K_{oc})	20–136[e]	72–136 [c]	High potential for leaching
Dissociation constant (pKa)	2.8[a]	2.6 (as DMA)[g]	Dissociates rapidly to anion at environmental pHs
Vapour pressure at 25 °C	1.87×10^{-2} mPa[a]	Not available—salts based on DMA salts dissociate to acid in water[d]	Low potential to volatize

[a] Health Canada, 2007; [b] U.S.EPA, 2005; [c] HSDB, 2005; [d] APVMA, 2006; [e] HSDB, 2015; [f] University of Hertfordshire, 2018; [g] Qurratu and Reehan, 2016

1.3 Exposure

As an herbicide, 2,4-D is deliberately applied to food crops. It can potentially be present as a residue in foods and is likely the main source of non-occupational exposure to 2,4-D for the general Canadian population (Health Canada, 2005a). Levels of 2,4-D in drinking water are often below the detection limit (DL). When detected, levels of 2,4-D in ambient and treated drinking were generally <1 µg/L (Health Canada, 2005a, 2007). Inhalation exposure is estimated to account for less than 2% of total intake (Kennepohl et al., 2010). Allocating a 20% source contribution to drinking water is deemed appropriate given its minor contribution as a source of exposure (Krishnan and Carrier, 2013).

Water monitoring data from the provinces and territories (municipal and non-municipal supplies), PMRA and Environment Canada (Environment and Climate Change Canada, 2017) (Appendix C) were available for 2,4-D and were used to estimate a level <1 µg/L for 2,4-D in Canadian drinking (Health Canada, 2007).

Data requested from the provinces and territories show that 2,4-D levels are below the method detection limit or MDL (which ranged from 0.005-1.000 µg/L) in most samples for groundwater, surface water, raw water, treated water or distribution water where monitoring occurred (Alberta Environment and Sustainable Resource Development, 2013; Ministère du Développement durable, de l'Environnement, de la Faune et des Parcs, 2013; New Brunswick Department of Health, 2013; Nova Scotia Environment, 2013; Ontario Ministry of the Environment, 2013; Manitoba Conservation and Water Stewardship, 2013; Saskatchewan Water Security Agency, 2013).

2,4-D was not detected in samples of raw or treated municipal drinking water in both Nova Scotia (n = 249, MDL = 0.05–0.1 µg/L) and New Brunswick (n = 16, MDL = 0.05 µg/L) (New Brunswick Department of Health, 2013; Nova Scotia Environment, 2013).

Between 2002 and 2013, 2,4-D was detected in 3% of the drinking water samples in Quebec; the mean concentration was 0.099 µg/L and the highest value was 0.75 µg/L (n = 6,412 from 214 distribution installations, mostly from surface water, MDL = 0.01–1.000 µg/L) (Ministère du Développement durable, de l'Environnement, de la Faune et des Parcs, 2013).

In Ontario, the maximum concentration of 2,4-D detected in treated surface and groundwater samples taken between 2006 and 2013 was 10 µg/L; the mean was 1.0 µg/L (n = 6,034, DL unspecified) (Ontario Ministry of the Environment, 2013).

In Manitoba, 2,4-D was detected in 13% of surface water in the environment (river, lake, stream) sampled between 2001 and 2012 and the maximum concentration was 8.4 µg/L (n = 1,860, DL = 0.005 µg/L) (Manitoba Conservation and Water Stewardship, 2013).

In Saskatchewan, 2,4-D concentrations were measured in surface, ground and treated drinking water samples collected between 2001 and 2012. Only one of 61 samples was above the DL of 0.1–0.5 µg/L (Saskatchewan Water Security Agency, 2013).

In Alberta, 2,4-D was detected at levels of 0.002–1.235 µg/L in 18.4% of treated drinking water samples (75% surface water, 25% groundwater) between 1995 and 2010 (n = 2332, DL = 0.005 µg/L) (Alberta Environment and Sustainable Resource Development, 2013).

Additional Canadian water monitoring data were also available from the literature. Between April and September of 2007, 19 sites from 16 watersheds across Canada were sampled, including 15 sites downstream from urban centres. 2,4-D was detected in 85% of the 150 samples taken. The mean, median and range of concentrations for all samples of 2,4-D were 172.1 ng/L, 52.7 ng/L and <0.47–1960 ng/L, respectively (Glozier et al., 2012). Ontario urban streams had significantly higher concentrations of 2,4-D compared with all other areas ($p < 0.001$). No seasonal differences in stream concentrations of 2,4-D were observed across the country. Samples collected after significant rain events had three-fold higher concentrations of 2,4-D when compared to non-event samples. In Prairie rivers, samples downstream from urban centres had a 1.6-fold increase in 2,4-D when compared with upstream samples (Glozier et al., 2012).

Four rivers from areas in Quebec where corn and soy are intensely cultivated were sampled for 2,4-D from 1993 to 2001 (Giroux, 2002). Annual 2,4-D mean and median concentrations were 0.027–0.504 µg/L and 0.02–0.263 µg/L, respectively, while yearly maximum concentrations were 0.2–4.1 µg/L (Giroux, 2002).

Between 1998 and 2002, 2,4-D was detected in 12% of Ontario surface water samples (n = 262) taken from the Don River and Humber River watersheds. The mean concentration was 0.13 µg/L (MDL = 0.1 µg/L) and the maximum concentration was 3.2 µg/L (Struger and Fletcher, 2007). 2,4-D was present in drinking water above the DL (0.05–0.1 µg/L) in only 1% (1/122) of the kitchen taps of homes sampled as part of the Ontario Farm Family Health Study (Arbuckle et al., 2006). Under Environment Canada's Great Lakes Surveillance Program, surface water samples were taken from all Great Lakes except Michigan between 1994 and 2000. 2,4-D concentration ranges were 2.3–14.5 ng/L, <0.40–84.4 ng/L, <0.29–1.4 ng/L, and <0.40 to 2.5 ng/L for lakes Ontario, Erie, Huron, and Superior, respectively (Struger et al., 2004).

Reservoirs and associated treated drinking water from Manitoba, Saskatchewan, and Alberta (total of 15 locations) were sampled for 2,4-D from 2003 to 2005 (Donald et al., 2007). All samples tested (n = 206) were above the limit of detection (LOD) of 0.47 ng/L. Drinking water had a mean annual concentration of 75 ng/L and a maximum of 589 ng/L, while reservoir water had a mean concentration of 123 ng/L and a maximum concentration of 1850 ng/L (Donald et al., 2007).

No information was found regarding 2,4-D levels in Canadian foods. According to the World Health Organization (WHO, 2003) "Available evidence indicates that residues of 2,4-D rarely exceed a few tens of µg/kg in food"; thus 2,4-D is unlikely to accumulate in food. Health Canada (2018a) has set maximum residue limits for a variety of food products (including fruits, vegetables, and animal tissues/organs) of 0.01–5 ppm of 2,4-D. Estimates of the dietary exposure to 2,4-D have been calculated using average consumption of different foods, average residue values on those foods over a 70-year lifetime and the different eating habits of the population at various stages of life. The general population, infants/children, and youth (ages 7–12) were estimated to have intakes of 0.12, 0.27, and 0.16 µg/kg body weight (bw) per day of 2,4-D from food, respectively (Health Canada, 2007).

In general, exposure to 2,4-D from air is considered to be low. 2,4-D is most often detected in the air immediately following application (Tuduri et al., 2006). Interim results from a 3-year national air surveillance program by the Canadian Atmospheric Network for Currently Used Pesticides (CANCUP) showed that atmospheric concentrations of 2,4-D varied within years and time periods, and by regional characteristics (Yao et al., 2008). Atmospheric samples were collected at eight sites (six agricultural, one wetland, one urban) across Canada in 2004 and 2005. Average concentrations were 10.0–730 pg/m^3 in 2004 and 59.4–193 pg/m^3 in 2005 (MDL = 1.4 pg/m^3). Levels of 0–5 ng/m^3 were measured in air following turf spraying of 2,4-D in three suburbs of Quebec City between 2001 and 2002 (Giroux and Therrien, 2005). Levels recorded in Saskatchewan between 1989 and 2002 were 190–2,730 pg/m^3; peak levels were recorded in June, followed by progressively decreasing levels (Tuduri et al., 2006). In Alberta, the Air Research Users Group of Alberta Environment reported that most concentrations were below 0.1 ng/m^3 and the maximum was 0.36 ng/m^3 (n = 4 locations; MDL = 0.05 mg/m^3) (Kumar, 2001).

2,4-D was not detected at any sampling site of the CANCUP surveillance study in 2005 (n = 8 locations, four samples per location, MDL = 0.2 ng/g) (Yao et al., 2008).

Most Canadians have very low urine levels of 2,4-D, as measured in Cycle 1 (2007–2009) and Cycle 2 (2009–2011) of the Canadian Health Measures Survey (CHMS) (Health Canada, 2013). In the CHMS, group geometric means of urinary 2,4-D were not calculated if more than 40% of the samples were below the DL of 0.2 µg/L. When the data were stratified by sex and age, geometric means could only be calculated for Cycle 2 males in the age groups of 6–11, 20–39 and 40–59 years, as well as the male total age group (3–79 years), and the range was 0.24–0.29 µg/L (95% confidence interval (CI): 0.20–0.39 µg/L).

The Ontario Farm Family Health Study and the Pesticide Exposure Assessment Pilot Study reported mean urinary 2,4-D concentrations of 1.0–40.8 µg/L in farm applicators (n = 126; 20% below the LOD), of 0.7–2.0 µg/L in farmer's wives (*n* = 125; 84% below the LOD), and of 0.7–2.9 µg/L in their children (n = 92; 70% below the LOD) (Arbuckle et al., 1999b, 2004, 2005, 2006; Arbuckle and Ritter, 2005).

2.0 Health Considerations

All pesticides, including 2,4-D, are regulated by Health Canada's PMRA. PMRA conducts extensive evaluations and cyclical reviews of pesticides, including unpublished and proprietary information, as well as foreign reviews by other regulatory agencies such as the United States Environmental Protection Agency (US EPA). As such, this health assessment is primarily based on PMRA's evaluations and supporting documentation (Health Canada, 2005a, 2005b, 2006, 2007, 2018b, 2018c). Additionally, any reviews and relevant literature available since PMRA's evaluations were completed were also considered.

2.1 Kinetics

The pharmacokinetics of 2,4-D is fairly consistent across species, with the exception of dogs which have a longer plasma half-life due to their slower renal transport clearance mechanism and their inability to excrete organic acids (van Ravenzwaay et al., 2003; Timchalk, 2004). Because of dogs' substantially higher body burden at comparable doses, canine data may not be relevant to human health risk assessment (Timchalk, 2004).

The pharmacokinetics of 2,4-D following dermal absorption is different from that following oral ingestion (Kennepohl et al., 2010). Given that dermal absorption is not a significant route of exposure via drinking water, pharmacokinetic studies using dermal exposure are not further considered. As the salts and ester compounds of 2,4-D are rapidly hydrolyzed to the acid in water after absorption (Frantz and Kropscott, 1993), only the pharmacokinetics of the acid form of 2,4-D will be discussed.

Absorption: 2,4-D is readily absorbed from the gastrointestinal tract (92–99%) following oral ingestion in rats and humans, with the rate of absorption decreasing with increasing dose (Sauerhoff et al., 1977; Gorzinski et al., 1987; Timchalk et al., 1990; Kennepohl et al., 2010). Absorption rate was unaffected by sex in a study using Sprague–Dawley rats orally dosed with 5 or 200 mg/kg of 2,4-D (Griffin et al., 1997).

In humans, peak plasma concentrations of 25–40 µg/mL were attained between 7 h and 24 h after capsules containing 5 mg/kg bw of 2,4-D were ingested by six healthy male volunteers (Kohli et al., 1974). In Sprague–Dawley rats given 5 mg/kg bw of 2,4-D orally, peak blood concentrations of 7.5 µg/L for males and 16.2 µg/L for females were reached in 26.8 min and 42.9 min, respectively (Griffin et al., 1997).

Distribution: Being very hydrophilic, 2,4-D rapidly distributes throughout the body but does not appear to accumulate in any tissue with repeated dosing (Erne, 1966; Munro et al., 1992; OEHHA 2009; Kennepohl et al., 2010). 2,4-D is highly bound (93 to 97 %) to plasma proteins over a broad range of concentrations (Timchalk, 2004). Distribution was similar in several species (mice, hamsters, rats, pigs, calves, chickens) that were orally administered 2,4-D with the highest values reported in the liver, kidney, lung and spleen. The values sometimes exceeded that found in plasma (Erne, 1966; Griffin et al., 1997). Low levels were also seen in the gonads, fat and brain (Griffin et al., 1997). Penetration of 2,4-D into adipose tissue and into the central nervous system was limited and likely influenced by 2,4-D existing predominantly in the ionized form at physiological pH making it unable to readily cross lipid membranes (Erne, 1966; Munro et al., 1992). Over time, the concentration of 2,4-D in the kidneys eventually exceeds the levels detected in the blood/plasma, reflecting the importance of the kidneys as the primary route of 2,4-D elimination (Timchaulk, 2004). In rats given single oral doses, 2,4-D levels in all tissues peaked at 6 hours and then dropped rapidly over 24 hours (Munro et al., 1992).

In animal studies using radiolabelled single doses, 2,4-D was reported to cross the placenta and was detected in the fetuses of rats and mice. However, elimination from the fetuses was rapid—within 24 h (Munro et al., 1992).

Metabolism: 2,4-D is not extensively metabolized in rats and humans regardless of the exposure dose, duration or route (Kohli et al., 1974; Munro et al., 1992). Only the parent compound was found in the urine and feces of rats after oral administration of 5–200 mg/kg bw of 2,4-D (Frantz and Kropscott, 1993; Griffin et al., 1997; van Ravenzwaay et al., 2003). In hamsters and mice, 2,4-D is the main compound found in urine, although conjugates (i.e., glycine and taurine in mice; glycine, taurine and glucuronide in hamsters) are also present at various levels (Griffin et al., 1997; van Ravenzwaay et al., 2003).

Elimination: Renal excretion is reported as the main route of 2,4-D elimination in humans, rats, hamsters and mice, while feces and expiration represent minor routes (Timchalk et al., 1990; Kennepohl et al., 2010). 2,4-D is secreted by the renal proximal tubules in rats and humans, using an active transport system that is saturable (Hasegawa et al., 2003; Nozaki et al., 2007; Kennepohl et al., 2010). In rats, saturation occurs at 50–60 mg/kg bw (Gorzinski et al., 1987; Hasegawa et al., 2003; Nozaki et al., 2007; Kennepohl et al., 2010). The rate of urinary excretion is inversely proportional to the dose administered (Kennepohl et al., 2010). In humans, the parent compound was detected in the urine as soon as 2 h following a single oral administration of 5 mg 2,4-D/kg bw, and 75% was excreted unchanged within 96 h (Kohli et al., 1974). The average half-life in humans following ingestion ranges from 18 h to 40 h, although a high value of 220 h has been reported (Friesen et al., 1990). The variability in half-life is likely related to differences in urine pH and its effect on renal clearance (Friesen et al., 1990). Rats given single oral doses of 10, 50, or 150 mg/kg bw of 2,4-D showed a biphasic clearance with mean excretion half-lives of 0.9 h for the alpha phase and of 18 h for the beta phase (Smith et al., 1990). Excretion reached saturation at 50 mg/kg bw (Smith et al., 1990; van Ravenzwaay et al., 2003). Female rats exposed to 200 mg/kg bw had a longer elimination half-life (139.4 h) than males (34.6 h) (Griffin et al., 1997).

2.2 Health effects

With very few exceptions (most notably DEA), the effects and relative toxicities of the salt and ester forms of 2,4-D are quite similar to those of the acid form (Health Canada, 2005a; US EPA, 2005).

2.3 Effects in humans

Although some epidemiological studies have shown associations between exposure to 2,4-D and the risk of cancer, birth defects, or Parkinson's disease, study shortcomings (i.e., improper exposure measurements, other confounding factors (such as co-exposure to other pesticides or contaminants, small sample sizes) hinder any definitive conclusions.

Information on acute toxicity is limited to cases of accidental and intentional ingestion of 2,4-D. These cases mostly involved ingesting mixtures containing 2,4-D in combination with other herbicides (e.g., dicamba, methylchlorophenoxyacetic acid) and/or with solvents and emulsifiers used in various formulations, making it difficult to differentiate the toxicity of 2,4-D from these other chemicals. Additionally, patients often vomit following ingestion. Both the ingestion of mixtures and the presence of vomiting could account for the wide range of oral LD_{50} values for humans (300–1000 mg/kg bw) available in the literature (Nielsen et al., 1965; Kancir et al., 1988; Friesen et al., 1990; Durakovic et al., 1992; Bradberry et al., 2000; Brahmi et al., 2003). Symptoms following oral ingestion of mixtures containing 2,4-D have been reported in a number of organs and organ systems, including the kidney, the central nervous system, the gastrointestinal tract and the cardiovascular system; death resulted from severe multiple organ failure or cardiac arrest in cases of fatal poisonings (O'Reilly, 1984; Kancir et al., 1988; Flanagan et al., 1990; Friesen et al., 1990; Durakovic et al., 1992; Keller et al., 1994; Bradberry et al., 2000; Brahmi et al., 2003). No signs of toxicity were observed in five healthy male volunteers given a single 5 mg/kg bw dose of analytical grade 2,4-D (Sauerhoff, 1977).

Cancer: Although some epidemiological studies have observed associations between exposure to 2,4-D and non-Hodgkin's lymphoma (NHL), soft-tissue sarcoma (STS), and prostate and gastric cancers in industrial and agriculture workers (Miligi et al., 2003, 2006; Mills et al., 2005; Mills and Yang, 2007), other studies have failed to support such associations (Wiklund et al., 1987; De Roos et al., 2003; Eriksson et al., 2008; Goodman et al., 2015, 2017). The absence of direct measures of individual exposure, the small cohort sizes and the presence of other contaminants, such as other pesticides, make it difficult to draw definitive conclusions. Moreover, the vast majority of the recent follow-up and meta-analysis studies have found no relationship between 2,4-D exposure and gastric, prostate or soft-tissue cancers (Bloemen et al., 1993; Kogevinas et al., 1997; Burns et al., 2011; Pahwa et al., 2011; Burns and Swaen, 2012; Goodman et al., 2017).

Finnish workers spraying 2,4-D exclusively did not have an increased number of chromosome aberrations in peripheral lymphocytes. The length of exposure ranged from 9 to 28 days; urinary levels of 2,4-D taken at the end of exposure ranged from 0.02 to 1.56 mg/L and showed large variability among individuals. The use of personal protective equipment was not specified (Mustonen et al., 1986).

Non-Cancer: Although a relationship between exposure to 2,4-D and Parkinson's disease has been suggested in the past these studies often looked at overall pesticide exposure rather than at a specific pesticide (Semchuk et al., 1992). With the exception of Tanner et al. (2009), most recent studies have not found an association between Parkinson's disease and exposure to 2,4-D (Kamel et al., 2007; Dhillon et al., 2008; Hancock et al., 2008; Rugbjerg et al., 2011; Burns and Swaen, 2012). In this occupational case–control study, a slight increase in the odds of Parkinson's disease and the use of 2,4-D in men from eight North American clinics was observed (OR = 2.59, 95% CI = 1.03–6.48); however, the study had a number of weaknesses, including a risk of recall bias, poorly characterized exposure, co-exposure of some subjects to other pesticides and a lower limit of the CI greater than 1.0 (Tanner et al., 2009).

Developmental and reproductive toxicity: The interpretation of epidemiological results for potential developmental and reproductive effects was often confounded by factors such as the use of pesticide formulations, the general grouping of 2,4-D with other pesticide classes or with all phenoxy herbicides, the use of indirect measures of exposure, unmeasured confounding factors and biases, and, in older studies, pesticide contamination with dioxins (Health Canada, 2007). In the Ontario Farm Family Health Study, a retrospective questionnaire-based study carried out in 2000 in farm couples, pre- and post-conception exposure to phenoxy herbicides did not increase the odds of spontaneous abortion in the first trimester (Arbuckle et al., 1999a). In a study by Garry et al. (1996), the odds of central nervous system, circulatory/respiratory, urogenital and muscular anomalies in newborns (n = 4,935) of pesticide applicators (n = 34,772) in Minnesota were significantly associated with combined chlorophenoxy herbicide/fungicide exposure (OR = 1.86, 95% CI = 1.69–2.05); no individual exposure data were available for 2,4-D.

In a poorly described study, sperm analysis (volume, count, mobility, morphology) of 32 male farm sprayers exposed to 2,4-D (mean urinary concentration of 9.02 mg/L of 2,4-D) revealed significant decreases in sperm motility and in the number of live sperm, as well as a significant increase in abnormal sperm morphology when compared with unexposed controls (n = 25; no detectable 2,4-D in urine); however, results were inconsistent across exposure periods and information on timing of urine and semen collection was missing (Lerda and Rizzi, 1991). In an *in vitro* study using spermatozoa from healthy volunteers, doses of ≥10 µM of 2,4-D resulted in dose-dependent decreases in total motility, progressive motility and capacitation in the presence of progesterone, while doses of ≥1 µM decreased the ability of sperm to penetrate viscous medium (Tan et al., 2016). Doses of up to 200 µM had no effect on sperm viability, capacitation without progesterone, or acrosome reactions (Tan et al., 2016). The authors hypothesized that 2,4-D could alter intracellular calcium concentrations and induce oxidative stress (Tan et al., 2016).

2.4 Effects in animals

Adverse effects of 2,4-D observed in subchronic and chronic animal studies included kidney, liver, and retinal toxicity, changes in body and organ weights (thyroid, kidney, adrenals), and alterations in blood chemistry and thyroid hormone levels (Serota et al., 1983a, 1983b; Gorzinski et al., 1987; Schultze, 1991a, 1991b; Jeffries et al., 1995; Charles et al., 1996b; Mattsson et al., 1997; Marty et al., 2013; Neal et al., 2017). There was no evidence of carcinogenicity from 2,4-D compounds in animals, and 2,4-D generally did not induce reproductive or developmental effects in rodents except at maternally toxic doses (Jeffries et al., 1995; Charles et al., 1996a; Kennephol et al., 2010; Marty et al., 2013; Pochettino et al., 2016).

2,4-D had moderate acute toxicity when given orally to animals (Carreon et al., 1983; Gorzinski et al., 1987). The oral LD_{50}values for 2,4-D were 607 and 726 mg/kg bw for male and female Fischer 344 (F344) rats, respectively (Gorzinski et al., 1987). Different forms of 2,4-D had similar or lower oral toxicity when considered as the active ingredient (a.i.), with rat values of 536 and 424 mg/kg bw a.i. in males and females, respectively for isobutyl ester and 619 and 490 mg/kg bw a.i. in males and females, respectively, for DMA (Gorzinski et al., 1987). The IPA salts were less acutely toxic, with oral LD_{50} values of 1,646 and 2,322 mg/kg bw for female and male rats, respectively (Carreon et al., 1983).

Kidney effects: Renal effects consisting of slightly altered clinical chemistry and kidney weights were seen in a 2-year chronic toxicity/oncogenicity study in which F344 rats

(60/sex/dose) were fed diets containing 0, 5, 75 or 150 mg/kg bw per day of technical grade 2,4-D (purity 96.4%) with an interim sacrifice of 10 rats/sex/dose at 12 months (Jeffries et al., 1995; Charles et al., 1996a). Animal survival was unaffected by treatment. At 2 years, kidney weights were unaffected in males, whereas females had a slight, statistically significant decrease at 150 mg/kg bw per day and a statistically significant increase in kidney to body weight ratio at ≥75 mg/kg bw per day. Decreased food consumption was seen in females at ≥75 mg/kg bw per day and in males at 150 mg/kg bw per day; it was accompanied by decreased weight gain. At ≥75 mg/kg bw per day, BUN was decreased in males only, while creatinine was increased in females only. At interim sacrifice, both males and females dosed with ≥75 mg/kg bw per day had degeneration of the proximal convoluted tubules, but no effects on kidney histopathology were noted in rats sacrificed at 2 years (Jeffries et al., 1995; Charles et al., 1996a).

A similar 2-year dietary study using F344 rats (60/sex/dose) dosed with 0, 1, 5, 15 or 45 mg/kg bw per day of 2,4-D showed no effect on clinical chemistry, gross pathology or survival (Serota, 1986). Relative body weight gain in high-dose females was significantly reduced at 12 and 24 months, although food consumption was decreased at 12 months only. Absolute and relative kidney weights were increased in both males (≥15 mg/kg bw per day) and females (all doses) at 2 years. Renal pelvic mineralization was seen in females starting at 15 mg/kg bw per day. Changes in kidney histopathology (increased incidence of brown tubular pigment and severity of fine cytoplasmic vacuolization in the renal cortex) were seen in both sexes starting at 5 mg/kg bw per day, although a review by an independent Pathology Working Group at Research Triangle Park involving re-sectioning and reading kidney slides from the study found no difference in tubular cell pigmentation between the dosed and control groups (Health Canada, 2005b). The Pathology Working Group also concluded that the nature of the pigment in all study animals was morphologically similar to that known to occur spontaneously in F344 rats of this age. The incidence and severity of pelvic mineralization was considered to be treatment-related in males at the 45 mg/kg bw and in females at 15 and 45 mg/kg bw (Health Canada, 2005b). In a 2-year dietary study, B6C3F1 female mice were given 0, 5, 150 or 300 mg/kg bw per day of 2,4-D while males were given 0, 5, 62.5 and 125 mg/kg bw per day (Jeffries et al., 1995; Charles et al., 1996a). Dose-related increases in absolute and relative kidney weights were noted in both sexes at the two highest doses and were associated with minimal alterations in the descending proximal tubules of the kidneys. Vacuolization of the proximal tubules was also observed at these doses.

The kidney was a target organ in an extended one-generation dietary study (preceding the publication of the Organisation for Economic Co-operation and Development (OECD) guideline 443) in which female and male Crl:CD rats (27/sex/dose; parental generation or P1) were fed 2,4-D at 0, 7, 21 or 40 mg/kg bw per day until the end of lactation and 0, 6, 17 or 45 mg/kg bw per day until 7 weeks post mating, respectively (Marty et al., 2013). Diets were continued in the offspring (F1) up to postnatal day (PND) 139, with interim sacrifices at PNDs 60, 70 and 90. Statistically significant increases in absolute and relative kidney weights were seen in high-dose P1 males and in high-dose F1 females at PND 139. Renal lesions were seen in high-dose P1 males, in mid-dose F1 males and in high-dose F1 animals (both sexes); they were characterized by very slight to slight degeneration of the proximal convoluted tubules. Lesions were more severe in males than females.

***Liver effects*:** In a 2-year chronic toxicity/oncogenicity study, F344 rats (60/sex/dose) were fed diets containing 0, 5, 75 or 150 mg/kg bw per day of technical grade 2,4-D (purity 96.4%); there was an interim sacrifice of 10 rats/sex/dose at 12 months (Jeffries et al., 1995;

Charles et al., 1996a). Animal survival was unaffected by treatment. In both sexes, liver weights were significantly decreased at the two highest doses while ALP was significantly increased. In males only, ALT and AST were significantly increased starting at ≥75 mg/kg bw per day. Histopathological examination showed minimal panlobular discolouration in the liver, although the authors did not consider the findings to be toxicologically significant.

Liver effects (changes in clinical chemistry and histology) have been reported for both F344 rats and B6C3F1 mice given dietary doses of up to 150 and 300 mg/kg bw per day of 2,4-D, respectively, for 13 weeks (Serota et al., 1983a, 1983b; Gorzinski et al., 1987; Schultze, 1991a, 1991b). Decreases in alanine transaminase (ALT)[1], aspartate transaminase (AST)[2], alkaline phosphatase (ALP) activities and BUN were observed in rats of both sexes exposed to 15 and 45 mg/kg bw per day for 13 weeks (Serota et al., 1983a). By contrast, in another 13-week study, rats receiving the top dose of technical grade 2,4-D (150 mg/kg bw per day) had a slight statistically significant increase in ALT in both sexes, while ALP and relative liver weights were slightly increased in females only (Gorzinski et al., 1987). Both sexes showed minor, nonspecific hepatocellular changes at the two highest doses (100 and 150 mg/kg bw per day) (Gorzinski et al., 1987). Similarly, ALT and AST levels increased in male and female rats exposed to 2,4-D at 100 and 300 mg/kg bw per day (doses ranged from 1-300 mg/kg bw per day for 13 weeks) and were accompanied by increased liver weight, liver lesions and centrilobular hepatocellular hypertrophy (Schultze, 1991b). Mice exposed to 1-300 mg/kg bw per day (both sexes) for 13 weeks also had histopathological lesions of the liver (characterized as nuclear hyperchromatism), and decreased glycogen in periportal hepatocytes but only at the highest dose given (Schultze, 1991a).

Endocrine effects: Increased thyroid weights, non-significant histopatholgical changes (parfollicular cell nodular hyperplasia), and decreased thyroxin (T4) levels were seen starting at 75 mg/kg bw per day in a 2-year chronic toxicity/oncogenicity study in F344 rats fed diets containing 0, 5, 75 or 150 mg/kg bw per day of 2,4-D (Jeffries et al., 1995; Charles et al., 1996a). T4 levels were also significantly decreased in the two highest dose groups of F344 rats (females only) fed diets containing 0, 15, 60, and 150 mg/kg bw per day of 2,4-D for 13-weeks (Gorzinski et al., 1987). In another 13-week F344 rat feeding study, absolute and relative thyroid weights were increased in males at all doses (1, 5, 15, or 45 mg/kg bw per day of 2,4-D) and in females at the three highest doses, while T4 levels were increased in males at 5 and 15 mg/kg bw per day (Serota et al., 1983a). In a comprehensive one-generation reproductive study in CD rats examining androgen, estrogen, and thyroid endpoints, endocrine-related effects were limited to slight thyroid hormone changes in pregnant dams only and were considered adaptive by the authors (Marty et al., 2013). Increased adrenal gland weight was observed in female mice at the 5 mg/kg bw per day and in F344 rats of both sexes at 100 and 300 mg/kg bw per day; changes in rats were correlated with cellular hypertrophy of the zona glomerulosa (Serota et al., 1983b; Schultze, 1991a).

Based on a recent comprehensive review of *in vitro* and *in vivo* studies, 2,4-D has a low potential to interact with the endocrine system (Neal et al., 2017). The review used a weight-of-evidence approach, and it had a detailed protocol for literature search and for inclusion and evaluation of the quality of both regulatory and published mammalian toxicological and

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[1]The terms ALT and SGPT (serum glutamic pyruvic transaminase) refer to the same parameter and are interchangeable.
[2] The terms AST and SGOT (serum glutamic oxaloacetic transaminase) refer to the same parameter and are interchangeable.

epidemiological studies. It also considered the coherence and consistency of findings and potential modes of action and it assessed the database for deficiencies. The review found no interactions between 2,4-D and endocrine pathways (estrogen, androgen, steroidogenesis or thyroid) (Neal et al., 2017). Results from five *in vitro* assays used by the US EPA's Endocrine Disruptor Screening Program were also negative for effects on androgen, estrogen, and steroidogenesis pathways of the endocrine system (Coady et al., 2014).

Eye effects: In subchronic and chronic feeding studies in F344 rats, s2-4-D and its esters caused histopathological lesions of the eyes (cataracts and retinal degeneration) at the highest doses tested (300 mg/kg bw per day in 13-week studies; 150 mg/kg bw per day in 1- and 2-year studies) (Schultze et al., 1991a; Szabo and Rachunek, 1991; Charles et al., 1996b; Mattsson et al., 1997).

Reproductive/developmental toxicity: In animal studies, reproductive effects were only observed at doses that exceeded renal clearance, while fetotoxic effects occurred only at maternally toxic doses (Charles et al., 2001; Marouani et al., 2017).
No evidence of reproductive toxicity or developmental neurotoxicity was found in an Extended One-Generation Reproductive Toxicity Study (pre-OECD 443) in which CD rats were fed 2,4-D (6-45 mg/kg bw per day) during critical windows of development (pre-mating, mating, gestation and lactation) (Marty et al., 2013).

Litter size and resorption rates were unaffected in a series of studies in pregnant rats (8–150 mg/kg bw per day as 2,4-D from GDs 6–15) and pregnant rabbits (10-90 mg/kg bw per day as 2,4-D from GDs 6–18) gavaged with 2,4-D, its salts and its esters (Charles et al., 2001). Significant fetal effects (decreased fetal body weights and increased fetal variations) were seen in rats given maternally toxic doses (≥90 mg/kg bw per day as 2,4-D) but not in rabbits. PMRA (Health Canada, 2007) considered both the maternal and the developmental no-observed-adverse-effect levels (NOAELs) for rats to be 25 mg/kg bw per day as 2,4-D.

No effects on body weight gain, number of pups born, pup postnatal mortality, or growth hormone levels were observed in pregnant rats given 70 mg/kg bw per day of 2,4-D in the diet from gestation day (GD) 16 until PND 23 or in their weanling pups fed the same diet until PND 45, 60, or 90 (Pochettino et al., 2016).

No signs of maternal toxicity were observed in pregnant CD-1 mice given a 2,4-D-amine derivative at 8.5, 37 or 370 mg/kg bw per day as 2,4-D in drinking water on GDs 6–16 although female pups had decreased body weight and minor reductions in the kidney weights at 37 and 370 mg/kg bw per day (Lee et al., 2001).

Although morphological changes in male reproductive organs, increased follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, and altered sperm number and motility were seen in male rats gavaged for 30 days with either100 or 200 mg/kg bw per day of 2,4-D, these effects occurred at doses that exceeded renal saturation (Marouani et al., 2017).

2.5 Genotoxicity and carcinogenicity

Negative results were obtained in the following in vitro studies: unscheduled DNA synthesis (UDS) assay using rat hepatocytes, sister chromatid exchange (SCE) assays using Chinese hamster ovary (CHO) cells, chromosomal aberration assays using human peripheral lymphocytes, apurinic/apyrimidine site activity assays using human fibroblasts and in Ames assays using several strains of *Salmonella typhimurium* both with and without metabolic activation (Linnainmaa, 1984; Mustonen et al., 1986; Clausen et al., 1990; Charles et al., 1999a; Gollapudi et al., 1999). Two in vitro studies were positive for genotoxic effects. Gonzalez et al.

(2005) reported dose-dependent increases in SCE and DNA-strand breaks in Chinese hamster ovary cells in a Comet assay. However, the study had a number of limitations: a weak dose–response relationship with no apparent time dependence, limited concentration range, a lack of positive control, and amalgamation of the untreated and vehicle control results (Gonzalez et al., 2005). An HGPRT locus assay using V79 Chinese hamster fibroblast cells showed 2,4-D was positive for gene mutation but only at high doses that were also cytotoxic (Pavlica et al., 1991).

Negative results were seen in two in vivo studies, sex-linked recessive lethal test and in a screening study testing for chromosome breakage and loss in *Drosophila melanogaster* fed 2,4-D (Woodruff et al., 1983; Zimmering et al., 1985). A few positive results have been reported in *Drosophila*, but these were observed at high doses and in unstable strains (Munro et al., 1992; Kaya et al., 1999). No chromosomal abnormalities or DNA lesions were observed in hepatocytes, lymphocytes or bone marrow cells of rats, mice and Chinese hamsters given 2,4-D orally and tested using bone marrow micronuclei, UDS, and SCE assays (Linnainmaa, 1984; Charles et al., 1999a, 1999b). A study by Amer and Aly (2001) did show chromosome aberrations in bone marrow and sperm head abnormalities in Swiss mice gavaged with 3.3 and 333 mg/kg bw of 2,4-D for 3 or 5 days; however, only 500 cells were counted and the increase in the percentage of abnormality was quite small.

Although some studies have given positive results, the overall lack of genotoxicity following *in vitro* and *in vivo* exposure to 2,4-D is consistent with its characteristics as a weak acid that is not significantly metabolized and is excreted rapidly from the body (Munro et al., 1992).

Neoplasms were not increased in a 2-year oncogenicity study where B6C3F1 mice were fed diets containing up to 300 mg/kg bw per day of 2,4-D (Jeffries et al., 1995; Charles et al., 1996a). Munro et al. (1992) describes an unpublished study in which the incidence of astrocytoma (brain cancer) was slightly increased in male rats given 45 mg/kg bw per day of 2,4-D; however, the characteristics of the tumours were atypical of a chemical carcinogen and repeat studies failed to confirm the results (Jeffries et al., 1995; Charles et al., 1996a; Kennepohl et al., 2010).

The International Agency for Research on Cancer (IARC) has classified 2,4-D in group 2B, “possibly carcinogenic to humans” based on limited evidence in animals, while other organizations have used a non-cancer approach to assess risk to human health from this contaminant (Loomis et al., 2015; IARC, 2017). The US EPA (2005, 2007) has repeatedly described 2,4-D as not classifiable with regard to human carcinogenicity. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) concluded that the carcinogenic potential of 2,4-D could not be evaluated on the basis of the available epidemiological studies and that 2,4-D and its salts and esters were not genotoxic (WHO, 2017). Moreover, in its re-evaluation for the continuing registration of this pesticide, Health Canada (2005a, 2007) concluded that 2,4-D is not carcinogenic, based on the absence of evidence of cancer in animals and on the lack of a clear association between exposure and cancer in human studies.

2.6 Mode of action

The kidney is the most sensitive target organ of 2,4-D toxicity (Gorzinski et al., 1987; Charles et al., 1996a, 1996b; Health Canada, 2007). Effects seen (increased relative and absolute kidney weights, changes in renal histopathology [particularly in the proximal tubules] and alterations in clinical chemistry) are consistent across species tested in both subchronic and chronic studies and are related to saturation of the renal clearance mechanism (Serota, 1983a, 1983b; Gorzinski et al., 1987; Schultze, 1991a; Munro et al., 1992; Jeffries et al., 1995; Charles et al., 1996a, 1996b).

In the kidney, 2,4-D accumulates in the renal proximal tubules through the action of a metabolically active renal organic anion transporter, OAT1 (Hasegawa et al. 2003; Timchalk, 2004; Nozaki et al. 2007; Burns and Swaen, 2012; Saghir et al., 2013). The OAT1 transporter plays a critical role in the dose-dependent systemic renal clearance of 2,4-D in rats and is saturated at oral gavage and dietary doses of approximately 50 mg/kg bw in male Fischer 344 rats given a single dose of 2,4-D (Gorzinski et al., 1987; Saghir et al., 2013). Sprague–Dawley rats given daily doses of 2,4-D reached renal saturation at 63 mg/kg bw per day for males (dosed for 71 days) and at 14 to 27 mg/kg bw per day for females (dosed for 96 days) (Saghir et al., 2013).

The mode of action for 2,4-D has not been clearly established. An increase in oxidative stress represents the most compelling evidence for the toxic mode of action of 2,4-D in animals, and seems to be responsible for the alterations observed in the kidney (degeneration of the proximal tubules, vacuolization of tubular cells and loss of the brush border) (Bongiovanni et al., 2011; Wafa et al., 2011). 2,4-D has been shown to perturb cell metabolism, deplete glutathione (GSH) reserves and thiol levels, and stimulate the peroxisome proliferator-activated receptor (PPAR). These precursor effects lead to an increase in reactive oxygen species (ROS) production that can induce kidney, liver and nervous system toxicity. It could also explain the genotoxic effects observed at high doses (Argese et al., 2005). An increase in markers of oxidation (hydroxyl radical, protein oxidation, carbonyl groups, lipid peroxides), a decrease in the GSH:GSSG (glutathione disulfide) ratio and in protein thiol content have been observed in rats exposed orally to 2,4-D (Ferri et al., 2007; Nakbi et al., 2012; Tayeb et al., 2012; Pochettino et al., 2013). These alterations were associated with a decrease in kidney and hepatic antioxidant enzyme activities, such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in rats. Wistar rats administered 15–150 mg/kg bw of 2,4-D butylglycol by gavage had an increase in hepatic lipid peroxidation and a decrease in catalase, glutathione peroxidase and glutathione reductase at all doses, and superoxide dismutase at high doses after 4 weeks, supporting the oxidative stress mode of action (Tayeb et al., 2013).

Moreover, *in vitro* studies using human and rat kidney cell lines and hepatocytes support the generation of ROS (Palmeira et al., 1995; Duchnowicz and Koter, 2003; Bharadwaj et al., 2005; Bukowska et al., 2008; Troudi et al., 2012).

2.7 Selected key study

In its re-evaluation for the continuing registration of 2,4-D (PACR2007-06), Health Canada's PMRA (2007, 2018b) identified the kidney as the most sensitive target organ across the database. Although no epidemiological studies have investigated the effects of 2,4-D on the kidney, kidney effects have been consistently observed in subchronic and chronic mice and rat studies (Serota et al, 1983a, 1983b; Serota, 1986; Gorzinski et al., 1987; Schultze, 1991; Jeffries et al., 1995). PMRA's assessment established an acceptable daily intake (ADI) of 0.017 mg/kg bw per day using a NOAEL of 5 mg/kg bw per day derived from two long-term dietary studies in rats that showed kidney effects, particularly degeneration of the descending proximal convoluted tubules (Serota, 1986; Jeffries et al., 1995; Health Canada, 2007, 2018). At the next highest dose level, kidney effects were noted (Health Canada, 2007, 2018). In addition, the selection of a NOAEL of 5 mg/kg bw per day as the point of departure for the determination of the ADI was supported by the NOAEL of 5 mg/kg bw per day established in the chronic mouse study in which kidney pathology (increased weight, degeneration with regeneration of descending limb of proximal tubule, decreased vacuolization and mineralization of proximal tubule, multifocal cortical cysts) occurred at the next dose level of 65 mg/kg bw per day (Health Canada, 2007, 2018).

3.0 Derivation of the human health reference value

As noted above, the NOAEL of 5 mg/kg bw per day for kidney effects in both rats and mice was selected as the basis for the current risk assessment. Using this NOAEL, the ADI (Health Canada, 2007, 2018b) was calculated as follows:

$$\text{ADI} = \frac{5 \text{ mg/kg bw per day}}{300}$$

$$= 0.017 \text{ mg/kg bw per day}$$

where:

- 5 mg/kg bw per day is the NOAEL, based on kidney effects; and
- 300 is the uncertainty factor, selected to account for interspecies variation (×10), intraspecies variation (×10), and potential sensitivity to the young, noted in a limited rat reproduction study and in a series of published neurotoxicity studies (×3).

Based on the ADI of 0.017 mg/kg bw per day, a health-based value (HBV) for 2,4-D in drinking water was derived as follows:

$$\text{HBV} = \frac{0.017 \text{ mg/kg bw per day} \times 74 \text{ kg} \times 0.20}{1.53 \text{ L/day}}$$

$$= 0.16 \text{ mg/L}$$

where:

- 0.017 mg/kg bw per day is the ADI calculated using a NOAEL of 5 mg/kg bw/day (Health Canada, 2007);
- 74 kg is the adult body weight (Health Canada, in preparation);
- 1.53 L per day is the daily volume of tap water consumed by an adult (Health Canada, in preparation);
- 0.20 is the default allocation factor for drinking water, since drinking water is not a major source of exposure to 2,4-D and there is evidence of the presence of 2,4-D in one of the other media (i.e., food) (Krishnan and Carrier, 2013).

4.0 Analytical and Treatment Considerations

4.1 Analytical methods to detect 2,4-D

Standardized methods available for the analysis of 2,4-D in source and drinking water and their respective method detection limits (MDL) are summarized in Table 2. MDLs are dependent on the sample matrix, instrumentation, and selected operating conditions and will vary between individual laboratories. The MDLs or method reporting limits (MRLs) from provincial and territorial data are in the range of 0.005 to 1.0 µg/L (Ministère du Développement Durable, de l'Environnement, de la Faune et des Parcs, 2019; Nova Scotia Environment, 2019; PEI Department of Communities, Land and Environment, 2019).

Drinking water utilities should discuss sampling requirements with the accredited laboratory conducting the analysis to ensure that quality control procedures are met and that MRLs are low enough to ensure accurate monitoring at concentrations below the maximum acceptable concentration (MAC). Sample processing considerations for the analysis of 2,4-D in drinking water (e.g. sample preservation, storage) can be found in the references listed in Table 2. In addition, Clausen (2000) reported that 2,4-D was retained on filters made of cellulose acetate, nylon or polyethersulfone materials when sampling or analyses involved filtering the water sample to separate suspended solids from solution. Filters made of polyvinylidene fluoride or polytetrafluorethylene materials were found to retain less 2,4-D.

Table 2. Standardized methods for the analysis of 2,4-D in drinking water

Method	Technique	MDL (µg/L)	Interferences
EPA 515.1 revision 4.1 (US EPA, 1995a)	Gas chromatography with electron capture detector (GC/ECD)	0.078	Alkaline substances; organic acids and phenols; plastic; analyzing a low concentration sample immediately after a high concentration sample
EPA 515.2 revision 1.1 (US EPA, 1995b)	Liquid–solid extraction (LSE) and GC/ECD	0.28	Alkaline substances; organic acids and phenols; plastic/phthalates
EPA 515.3 revision 1.0 (US EPA, 1996)	Liquid–liquid extraction, derivatization (LLED) and GC/ECD	0.35[a] to 0.36[b]	Analyzing a low concentration sample immediately after a high concentration sample; plastic/ phthalates; presence of water from base promoted esterification procedure
EPA 515.4 revision 1.0 (US EPA, 2000)	Liquid–liquid microextraction, derivatization (LLMED) and GC/ECD	0.055 to 0.066[c]	Plastic/phthalates; sodium sulphate
EPA 555 revision 1.0 (US EPA, 1992)	High performance liquid chromatography with photodiode array ultraviolet detector (HPLC/PDA-UV)	0.34 to 1.3[c]	Sodium sulphate
ASTM D5317-98 (Reapproved 2011) (ASTM, 2011)	GC/ECD	0.2	Alkaline substances; organic acids and phenols; phthalate esters (e.g., flexible plastics); analyzing a low concentration sample immediately after a high concentration sample
Standard Method 6640B or online version 6640B-01 (APHA, 2005, 2012, 2017)	Liquid–liquid microextraction, derivatization (LLMED) and GC/ECD	0.06 to 0.07[c]	Phthalate esters; avoid the use of plastics in the laboratory

[a] Using diazomethane
[b] Using the base promoted esterification procedure
[c] MDLs are instrument- and matrix-dependent.

4.2 Treatment considerations

Treatment technologies are available to effectively decrease 2,4-D concentrations in drinking water. Activated carbon adsorption is recognized as the best available technology for 2,4-D removal (US EPA, 2009b). Biological filtration processes can also decrease 2,4-D concentrations; however, conventional treatment is not effective for 2,4-D removal. Typical disinfection processes used in drinking water treatment also have limited potential to reduce 2,4-D concentrations. At the residential scale, certified treatment devices are available for the removal of 2,4-D. These devices rely mainly on adsorption (activated carbon) and reverse osmosis technologies.

4.2.1 Municipal-scale treatment

Since 2,4-D concentrations are low in source water, treatment technology data reported in the literature generally have low influent concentrations (<10 µg/L). Information on the removal efficiencies and operational conditions from these studies is reported below, as the studies provide an indication of the effectiveness of specific treatment technologies for 2,4-D removal. The selection of an appropriate treatment process for a specific water supply will depend on many factors, including the raw water source and its characteristics, the operational conditions of the selected treatment method and the utility's treatment goals.

4.2.1.1 Conventional treatment

Conventional drinking water treatment processes, such as chemical coagulation, clarification, rapid sand filtration and chlorination, are reported to be ineffective in decreasing the concentration of a variety of classes of pesticides, including polar pesticides like phenoxyacetic acids (Robeck et al., 1965; Miltner et al., 1989; Croll et al., 1992; Haist-Gulde et al., 1993; Frick and Dalton, 2005; Chowdhury et al., 2010; Hughes and Younker, 2011). Biological filtration (see section 4.2.1.4) has shown some capacity for removing chlorophenoxy acids (Foster et al., 1991, 1992) and 2,4-D specifically (Woudneh et al., 1996, 1997; Storck et al., 2010; Zearley and Summers, 2012, 2015; Huntscha et al., 2013).

4.2.1.2 Oxidation and hydrolysis

Chamberlain et al. (2012) conducted bench-scale tests to assess what degradation of pesticides could be expected to occur naturally in the environment by hydrolysis/photolysis and by disinfection processes typically used in drinking water treatment. Common oxidation/ disinfection processes were evaluated as outlined in Table 3. Laboratory experiments were conducted at 23 ± 1°C at pHs of 6.6 and 8.6 and an initial 2,4-D concentration of 25 µg/L. Solutions were spiked with an oxidant or subjected to UV photolysis at 254 nm at the doses noted in Table 3. Reaction media for the hydrolysis experiments were sodium-phosphate-buffered laboratory water at pHs 2, 7 and 12. Removal of 2,4-D was low in all experiments.

The ozone (O_3) findings in Table 3 are consistent with predicted removal based on O_3 rate constant studies for 2,4-D at typical drinking water exposures (Yao and Haag, 1991; Xiong and Graham, 1992; Hu et al., 2000; Benitez et al., 2004; Giri et al., 2007). Meijers et al. (1995) reported similar low reductions of 2,4-D using typical O_3 dosages (reported as the O_3 to dissolved organic carbon [DOC] ratio) in bench-scale experiments at 5°C (pH 7.2, O_3:DOC = 0.53, 2,4-D influent concentrations = 0.9–6.4 µg/L). However, 2,4-D removal increased to 48% at 20°C (pH 7.2, O_3:DOC = 0.55) and 74% when the pH and O_3 dose were increased (pH 8.3, O_3:DOC = 0.95).

Table 3. Degradation of 2,4-D via oxidation

Process	Dose used	CT[a] Range (mg·min/L)	Decrease (%)
Free chlorine	2–5 mg/L	107–173	<20
Monochloramine	9–14 mg/L	1,287–1,430	<20
Permanganate	3–5 mg/L	134–164	<20
Chlorine dioxide	2–3 mg/L	38–73	<20
Hydrogen peroxide	100 mg/L	933–1,100	<20
Ozone	1–2 mg/L	0.2–0.3	<20
UV_{254}	77–97 mV·s/cm^2		<20

[a] CT = disinfectant concentration (C) × time (T)
Source: Chamberlain et al. (2012)

The UV findings in Table 3 are also consistent with pilot-plant experiments conducted by Kruithof et al. (2002) and bench-scale experiments conducted by Benitez et al. (2004). Kruithof et al. (2002) reported a 58% decrease in 2,4-D at a UV dose of 2,000 mJ/cm^2, much higher than needed for disinfection. Benitez et al. (2004) reported a decrease of 30–40% following 100 min of reaction time at pHs 9 and 7, respectively (initial 2,4-D concentration of 50 mg/L) using a low-pressure UV lamp.

4.2.1.3 Adsorption

Activated carbon adsorption is a widely used technology to reduce the concentration of micropollutants in drinking water (Haist-Gulde and Happel, 2012; van der Aa et al., 2012). Activated carbon can be applied in two ways: slurry applications using powdered activated carbon (PAC) or fixed-bed reactors with granular activated carbon (GAC) (Chowdhury et al., 2013).

Powdered activated carbon

Many pesticides have been found to strongly adsorb to PAC (Chowdhury et al., 2013). The use of PAC offers the advantage of providing virgin carbon when required (e.g., during the pesticide application season) (Miltner et al., 1989). The capacity of PAC to remove pesticides by adsorption depends on the PAC dose, the contact time, the PAC characteristics (type, particle size), the adsorbability of the contaminant and the competition for adsorption sites from natural organic matter (NOM) (Haist-Gulde and Happel, 2012).

Although there is a lack of published literature regarding the use of PAC in drinking water treatment specifically for 2,4-D adsorption, based on its chemical properties and published research on adsorption technology, PAC is expected to adequately remove 2,4-D (Haist-Gulde, 2014). As removal efficiency varies by PAC type, adsorption kinetics should be considered when optimizing PAC systems (Gustafson et al., 2003; Summers et al., 2010). Jar tests, with varied carbons and conditions, are generally used to develop percentage-removal-versus-PAC-dosage curves.

Increasing the PAC dose will increase the removal percentage for a contaminant but not in a proportional ratio (Robeck, 1965; Miltner et al., 1989; Gustafson et al., 2003; Westerhoff et al., 2005; Chowdhury et al., 2013). Increasing the contact time will increase the amount of pesticide removed, as the time to equilibrium (i.e., contact time needed to utilize the full capacity of the PAC) is generally greater than that used by water treatment plants (Gustafson et al., 2003).

Decreasing the PAC size reduces the time to equilibrium. However, the benefit from decreasing the PAC size is typically offset by the difficulty in removing the smaller PAC from solution (Chowdhury et al., 2013).

Generally, the presence of NOM adds complexity to PAC treatment because the NOM competes directly for adsorption sites or fouls the PAC by blocking pores (Chowdhury et al., 2013). Robeck et al. (1965) found that a PAC dose of 29 ppm was required to reduce lindane concentrations from 10 ppb to 1 ppb in river water (chemical oxygen demand: 5–35 ppm) compared with a dose of 2 ppm in distilled water. This was attributed to the NOM found in the river water. Responsible authorities should be aware of the impact of NOM on PAC systems, as it may impact water quality objectives for 2,4-D removal. In addition, PAC dosage for taste and odour control, a common application of PAC, may be a fraction of that required for 2,4-D removal.

When PAC is added prior to some treatment processes, it can interact with water treatment chemicals, leaving less PAC available to lower concentrations of contaminants such as 2,4-D (Greene et al., 1994; Summers et al., 2010). Kouras et al. (1998) reported that the addition of PAC before coagulant dosing increased the required PAC dose from 20 mg/L to 40 mg/L to reach a target concentration of 0.1 µg/L for lindane. This was attributed to the PAC particles becoming incorporated into the floc structure, thereby reducing available adsorption sites.

<u>*Granular activated carbon*</u>

The use of GAC is an effective approach for treating organic contaminants that are regularly found in source water at concentrations of concern (Chowdhury et al., 2013). According to the WHO (2011), GAC treatment should be able to decrease 2,4-D concentrations to 1 µg/L. The capacity of GAC to remove pesticides by adsorption depends on the filter velocity, the empty bed contact time (EBCT), the GAC characteristics (type, particle size, reactivation method), the adsorbability of the contaminant, the filter run time and the competition from NOM for adsorption sites (Haist-Gulde and Happel, 2012).

A pilot-scale study demonstrated the capacity of GAC to remove 2,4-D (Haist-Gulde and Happel, 2012). Three GAC fixed-bed adsorbers were installed in parallel at a surface water treatment plant and each was filled with a different bituminous coal carbon. The pilot plant was operated for 21 months under typical conditions, and breakthrough curves were measured for ionic pesticides and metabolites, including 2,4-D. Feed water was taken from the GAC filter influent for the full-scale surface water treatment plant (DOC = 1.3 mg/L) and spiked with 1 µg/L of 2,4-D. The breakthrough of 2,4-D was measured at bed depths of 0.5 m, 1.0 m, and 1.5 m, corresponding to EBCTs of 3 min, 6 min, and 9 min, respectively, at a filter velocity of 10 m/h. Complete removal was possible to about 50,000 bed volumes (BVs) at a 1.5 m bed depth (Haist-Gulde and Happel, 2012), although the performance of the three carbons varied. Reported BVs treated to 10% breakthrough were 37,000, 56,000 and 73,000 (at a 1.5 m bed depth) for carbons 1, 2 and 3, respectively. The impact of bed depth was also evaluated; the operation time to 20% breakthrough ranged from approximately 30,000 to 80,000 BVs for bed depths of 0.5 m and 1.5 m, respectively. Performance at a 1.0 m bed depth was typically found to be slightly better than at 0.5 m. The study concluded that 2,4-D should be removed by most GAC adsorbers; short GAC bed depths are not suitable for the removal of ionic pesticides such as 2,4-D; and GAC selection pilot studies are required (Haist-Gulde and Happel, 2012).

Because GAC fixed-bed adsorbers are typically operated on a continuous basis, NOM adsorbs on the GAC during periods when contaminants such as 2,4-D are absent from the source water. The GAC becomes fouled (or preloaded) with NOM and the rate of adsorption for targeted contaminants such as 2,4-D is adversely affected. If the GAC is exhausted, it may be completely or partially ineffective for 2,4-D removal (Knappe et al., 1999; Summers et al., 2010; Haist-Gulde and Happel, 2012; Chowdhury et al., 2013).

Responsible authorities should be aware of the impact of NOM on GAC systems, as it may impact water quality objectives for 2,4-D removal. DOC monitoring is recommended as a conservative tracer for contaminant breakthrough (Corwin and Summers, 2012; Kennedy et al., 2012).

4.2.1.4 Biological filtration

Biological filtration processes include slow sand filtration, riverbank filtration and engineered biological filtration. With slow sand filtration, the significant removal mechanism is biodegradation (Woudneh et al., 1997). With riverbank filtration, removal mechanisms include adsorption, biodegradation and transport processes such as convection, diffusion and dispersion (Storck et al., 2010). However, adsorption plays a minor role for polar organic compounds such as 2,4-D (Huntscha et al., 2013). Engineered biofiltration is a modification of conventional filtration whereby the filter media (i.e., anthracite/sand or GAC) has been allowed to develop a microbial biofilm that assists in the removal of fine particulate and dissolved organic materials (Symons et al., 2000). The filter media influences the removal mechanism, and both adsorption and biodegradation can occur when using GAC (van der Aa et al., 2012), whereas biodegradation occurs with anthracite/sand (Zearley and Summers, 2012, 2015). Although biological filtration has the ability to decrease 2,4-D concentrations, Benner et al. (2013) noted that the removal mechanisms remain largely unknown. The authors recommended more research to fill knowledge gaps and enable process optimization for the removal of micropollutants, including those that are less biodegradable and those that are present on an intermittent basis.

Slow sand filtration

A pilot-scale study conducted in the United Kingdom demonstrated the capacity of slow sand filtration to remove 2,4-D (Woudneh et al., 1996, 1997). This study dealt with both the seasonal variation (Woudneh et al., 1996) and the impact of filter maturity (Woudneh et al., 1997) on 2,4-D removal. In brief, two filter bed depths (300 mm and 500 mm) and two flow rates (0.06 m/h and 0.12 m/h) were studied over 15 months in three study periods: 1) October to December 1994, 2) March 1995, and 3) August and September 1995.

For the first study period, removals of approximately 90% were observed, as summarized in Table 4.

Table 4. Effect of flow rate, bed depth and contact time on the removal of 2,4-D by slow sand filtration[a,b]

Filter bed depth (mm)	Flow rate (m^3/h)	Contact time (h)	Removal efficiency (%)
300	0.06	4.3	89.6[c]
	0.12	2.15	90.5[d]
500	0.06	7.16	93.3[c]
	0.12	3.58	91.6[d]

[a] Adapted from Woudneh et al. (1996)
[b] Influent concentrations of 6–10 μg/L
[c] Average of three independent measurements conducted October–December 1994
[d] Average of five independent measurements conducted October–December 1994

In the second study period, the filter beds were cleaned, the sand was replaced and the percentage removal dropped to zero. After 20 days, adequate biological growth was restored to achieve complete removal of 2,4-D. The authors cautioned that re-establishing the biological growth within a filter bed depends on a number of factors, such as the chemical/microbiological water quality and environmental conditions. Thus, the time to restore filter performance for the removal of 2,4-D will be site specific (Woudneh et al., 1997).

During the third study period, the authors found 100% breakthrough of 2,4-D. Woudneh et al. (1996) hypothesized that the reduction in performance was due to either 1) a change in the aerobic nature of the filter bed, caused by decreased dissolved oxygen content of the water, or 2) a change in NOM, measured by a significant reduction in UV absorbance (at 254 nm) during August and September. Lower UV absorbance suggests the presence of more hydrophilic NOM, which can be easily consumed by microorganisms. The authors suggested that the presence of this nutrient source (e.g., hydrophilic NOM) caused the microorganisms to not biodegrade the 2,4-D. Complete removal of 2,4-D returned in October when the UV absorbance increased, suggesting the presence of more hydrophobic NOM. Hydrophobic NOM tends to be more recalcitrant, so 2,4-D becomes the preferred nutrient source for the microorganisms. Pilot testing is recommended to ensure that slow sand filtration will successfully treat a source water (Bellamy et al., 1985a, 1985b; Logsdon et al., 2002).

Riverbank filtration

Natural attenuation through riverbank filtration is one of the most basic and inexpensive methods of water treatment (Verstraeten and Heberer, 2002; Sørensen et al., 2006).

Huntscha et al. (2013) conducted field tests to develop 2,4-D breakthrough curves for a riverbank filtration site with short travel times (a few days) and oxic groundwater conditions most of the time. *In-situ* microbial reaction rates were measured for nine micropollutants, including 2,4-D. The test was conducted at >16°C with an initial 2,4-D concentration of 100 ng/L. First-order rate constants for 2,4-D degradation ranged from 0.1/h to 1.3/h, corresponding to a half-life of 0.5–6.7 h for 2,4-D. The results from two other riverbank filtration field studies with suboxic and anoxic groundwater conditions are summarized in Table 5. High removal efficiencies were observed at both sites. Chemical structure and physiochemical properties of a compound, retention time and redox setting are the key factors controlling the removal of a micropollutant (Storck et al., 2012).

Table 5. Degradation of 2,4-D in suboxic and anoxic conditions[a]

Initial concentration (µg/L)	Redox milieu	Residence time (Days)	2,4-D removal efficiency (%)	Dilution (%)
0.095	Suboxic	<1–3	86	None
0.3	Anoxic	14	>97	20

[a] Adapted from Storck et al. (2010)

Engineered biological filtration

Two bench-scale studies have demonstrated the capacity of engineered biofilters to remove 2,4-D (Zearley and Summers, 2012, 2015). In both studies, biofilters were constructed using biologically active sand, taken from a full-scale drinking water treatment plant where the source is impacted by upstream wastewater discharges and agricultural/urban runoff. The sand media had an effective size of 0.45 mm and an approximate uniformity coefficient of 1.3.

Feedwater was dechlorinated municipal water (pH = 7.7, alkalinity = 40 mg/L as $CaCO_3$), supplemented with dissolved organic matter to a target total organic carbon concentration of 3 mg/L. Removal rates in the order of 30–49% were observed in both studies during the acclimation periods (100–159 days).

In the first study, the long-term removal of 2,4-D under different EBCTs was assessed. The study was conducted at lab temperature (20 ± 2°C) and consisted of two columns in series operated at a hydraulic loading rate of 2.4 m/h to achieve a target EBCT of 7.5 min in the top column and 7.5 min in the bottom column (total ~ 15 min). The influent was spiked with 13 micropollutants (2,4-D influent concentration of 171 ± 57 ng/L) and operated for 350 days. Steady-state removal of 2,4-D was reported to be 68 ± 11% at an EBCT of 7.9 min and 77% ± 13% at an EBCT of 15.8 min (Zearley and Summers, 2012).

In the second study, seven biofilters were used to assess the effects of various intermittent loading conditions (i.e., periods with no 2,4-D spiking). The target hydraulic loading rate of the biofilters was 2.2 m/h to achieve an EBCT of 8.7 min with an influent 2,4-D spike of 87 ng/L ± 7.6 ng/L. In the control biofilter (constant 2,4-D loading), a steady-state removal of 70 ± 3.8% at an average EBCT of 8.2 min was reported. For the remaining six biofilters, 2,4-D spiking was initially conducted for 60–96 days, followed by periods of no spiking for five different time periods: 0, 36, 83, 149, and 263 days. Biomass acclimation was observed in the intermittently loaded biofilters with removal rates of 35–55%. Following 209 days of biofilter operation, greater than 70% removal was reported (regardless of no spiking for a period of 149 days), indicating that constant exposure to 2,4-D was not necessary for biomass acclimation. Adapted microorganisms were capable of biodegrading 2,4-D following non-exposure periods of up to 5 months; however, following 9 months of non-exposure, 2,4-D removal was significantly lower (approximately 20%) (Zearley and Summers, 2015).

4.2.1.5 Membrane filtration

Limited scientific literature has been published on the effectiveness of membrane treatment for the removal of 2,4-D from drinking water. Edwards and Schubert (1974) conducted bench-scale experiments to evaluate the removal rate of 2,4-D from an aqueous solution using cellulose acetate reverse osmosis membranes at a pressure of 50 psi. The rejection of 2,4-D from 10 mL samples initially varied between 52% and 65% but declined rapidly with successive 10 mL samples. Plakas and Karabelas (2012) reviewed the removal of a variety of pesticides from water by nanofiltration and reverse osmosis membranes. Although 2,4-D was not studied specifically, rejection rates varied from >10% to 100%, depending on the pesticide and the membrane. More hydrophobic and polar pesticides, such as 2,4-D, tend to have lower rejection due to adsorption and diffusion through the membrane, as well as polar interactions with charged membranes.

4.2.1.6 Combined treatment processes

Donald et al. (2007) investigated the occurrence of 45 pesticides in 15 untreated surface water reservoirs in Manitoba, Saskatchewan and Alberta from May 2003 to April 2004. The authors subsequently evaluated the effectiveness of treatment associated with the reservoirs in July 2004 and July 2005. Mean concentrations for 2,4-D in untreated water ranged from 12 ng/L ± 6 ng/L to 597 ng/L ± 199 ng/L. The overall mean for all sites was reported as 123 ng/L (n = 163). In July 2004 and July 2005, 28 simultaneous raw and treated water samples were collected to assess pesticide reduction by the water treatment plants associated with each surface water reservoir. Treatment processes varied at the 15 sites and included both conventional and advanced processes. Thirteen facilities had sand filtration, one had membrane filtration and one had no filtration. All facilities had chlorination, with 2 facilities also adding ammonia for chloramination; 8 facilities used potassium permanganate, 3 facilities used lime–soda ash and carbon dioxide, and 12 facilities used some form of activated carbon. The authors reported a 39% mean reduction in 2,4-D concentrations (data not provided). The authors concluded that the decrease in concentrations for the 29 pesticides that were detected during the study period (including 2,4-D) was highly variable and showed no obvious relation to differences in treatment processes. As the study design was not intended to characterize treatment efficacy, it is unknown which treatment processes contributed to the reduction in 2,4-D concentrations reported in this study.

4.2.1.7 Advanced oxidation processes

Advanced oxidation processes (AOPs) use combinations of oxidants, UV irradiation and catalysts to generate hydroxyl free radicals in water to make oxidation-reduction reactions more rapid or complete (Foster et al., 1991; Symons et al., 2000).

<u>Ozone and hydrogen peroxide:</u> Limited scientific literature has been published on the effectiveness of O_3 and hydrogen peroxide (O_3/H_2O_2) for the removal of 2,4-D from drinking water. Meijers et al. (1995) conducted bench-scale experiments and found that 2,4-D was degraded by 88–95% when ozonation was preceded by H_2O_2 dosage (O_3/DOC = 1.4; H_2O_2/O_3 = 0.5; pH 7.2–8.3; temp = 20ºC); pH was shown to have a minor effect on pesticide degradation using AOPs.

<u>UV and hydrogen peroxide:</u> Wols and Hofman-Caris (2012) reviewed the photochemical reaction constants required for UV AOPs and predicted low reduction for 2,4-D (~30%) using low-pressure UV lamps (400 mJ/cm^2). Benitez et al. (2004) found that a combined UV/H_2O_2 process increased the rate of 2,4-D removal compared with UV treatment alone in bench-scale experiments. However, 20–40 min were required to achieve 80% reduction. Initial concentrations were high (50 mg/L) and the results did not include information on the final treated levels. Alfano et al. (2001) conducted bench-scale experiments (initial 2,4-D concentration = 30 mg/L) and found that a combined UV/H_2O_2 process was 20 times more effective than UV treatment alone.

4.2.2 Residential-scale treatment

In cases where 2,4-D removal is desired at the household level, for example when a household obtains its drinking water from a private well, a residential drinking water treatment unit may be an option for decreasing 2,4-D concentrations in drinking water. Before a treatment unit is installed, the water should be tested to determine the general water chemistry and 2,4-D concentration in the source water. Periodic testing by an accredited laboratory should be conducted on both the water entering the treatment unit and the treated water, to verify that the treatment unit is effective. Units can lose removal capacity through use and time and need to be maintained and/or replaced. Consumers should verify the expected longevity of the components in the treatment unit according to the manufacturer's recommendations and service it when required.

Health Canada does not recommend specific brands of drinking water treatment units, but it strongly recommends that consumers use units that have been certified by an accredited certification body as meeting the appropriate NSF International Standard/American National

Standard Institute (NSF/ANSI) for drinking water treatment units. These standards ensure the material safety and performance of products that come into contact with drinking water. Certification organizations provide assurance that a product conforms to applicable standards and must be accredited by the Standards Council of Canada (SCC). Accredited organizations in Canada (SCC, 2019) include:

- CSA Group (www.csagroup.org);
- NSF International (www.nsf.org);
- Water Quality Association (www.wqa.org);
- UL LLC (www.ul.com);
- Bureau de Normalisation du Québec (www.bnq.qc.ca); and
- International Association of Plumbing and Mechanical Officials (www.iapmo.org).

An up-to-date list of accredited certification organizations can be obtained from the SCC (www.scc.ca).

A number of certified residential treatment devices are currently available for the removal of 2,4-D from drinking water. These devices rely on adsorption (activated carbon) and reverse osmosis technologies. Treatment devices to remove 2,4-D from untreated water (such as a private well) can be certified either specifically for 2,4-D removal or for the removal of volatile organic chemicals (VOCs) as a group. Drinking water treatment devices can be installed at the faucet (point-of-use) or at the location where water enters the home (point-of-entry) in residential settings to reduce contaminant concentrations.

For a drinking water treatment device to be certified to NSF/ANSI Standard 53 (Drinking Water Treatment Units–Health Effects) for 2,4-D removal, it must be capable of decreasing an average influent concentration of 0.210 mg/L to a maximum final (effluent) concentration of 0.07 mg/L. For a drinking water treatment device to be certified to the same standard for the removal of VOCs, the device must be capable of removing 2,4-D from an average influent concentration of 0.110 mg/L by greater than 98% to a maximum final (effluent) concentration of 0.0017 mg/L (NSF/ANSI, 2018a).

For a drinking water treatment device to be certified to NSF/ANSI Standard 58 (Reverse Osmosis Drinking Water Treatment Systems) for the removal of VOCs, the device must be capable of reducing 2,4-D from an average influent concentration of 0.110 mg/L by greater than 98% to a final (effluent) concentration of 0.0017 mg/L (NSF/ANSI, 2018b). Water that has been treated using reverse osmosis may be corrosive to internal plumbing components. Therefore, these units should be installed only at the point-of-use (POU). Also, as large quantities of influent water are needed to obtain the required volume of treated water, these units are generally not practical for point-of-entry installation.

5.0 Management strategies

All water utilities should implement a risk management approach, such as the source-to-tap or water safety plan approach, to ensure water safety (CCME, 2004; WHO, 2011, 2012). These approaches require a system assessment to characterize the source water, describe the treatment barriers that prevent or reduce contamination, identify the conditions that can result in contamination, and implement control measures. Operational monitoring is then established, and operational/management protocols are instituted (e.g., standard operating procedures, corrective actions and incident responses). Compliance monitoring is determined and other protocols to validate the water safety plan are implemented (e.g., record keeping, consumer satisfaction).

Operator training is also required to ensure the effectiveness of the water safety plan at all times (Smeets et al., 2009).

5.1 Monitoring

2,4-D can be present in groundwater and surface water in areas where it is being used depending on the type and extent of its application, environmental factors (e.g., amount of precipitation, soil type, hydrogeological setting, etc.) and environmental fate (e.g., mobility, leaching potential, degradation, etc.) in the surrounding area. Water utilities should consider the potential for 2,4-D to enter source water (e.g., raw water supply to the drinking water system) based on site-specific considerations.

When it is determined that 2,4-D may be present and monitoring is necessary then surface and groundwater sources should be characterized to determine the concentration of 2,4-D. This should include monitoring of surface water sources during periods of peak use and rainfall events and/or monitoring of groundwater annually. Where baseline data indicate that 2,4-D is not present in source water, monitoring may be reduced.

Where treatment is required to remove 2,4-D, operational monitoring should be implemented to confirm whether the treatment process is functioning as required. The frequency of operational monitoring will depend on the treatment process. For example, when using activated carbon, routine monitoring for dissolved organic carbon may act as an indicator of contaminant breakthrough. Responsible authorities should be aware of the impact of natural organic matter on activated carbon systems, as it may impact water quality objectives for 2,4-D removal.

Where treatment is in place for 2,4-D removal, compliance monitoring (i.e., paired samples of source and treated water to confirm the efficacy of treatment) should be conducted when routine operational monitoring indicates the potential for contaminant breakthrough or, at a minimum, on an annual basis.

6.0 International Considerations

This section presents drinking water guidelines, standards and/or guidance from other national and international organizations. Variations in these values can be attributed to the age of the assessments or to differing policies and approaches, including the choice of key study and the use of different consumption rates, body weights and source allocation factors (Table 6).

The U.S EPA (1991, 2005, 2010) established a drinking water maximum contaminant level (MCL) and a maximum contaminant level goal (MCLG) at 0.07 mg/L (70 µg/L) based on a reference dose (RfD) of 0.005 mg/kg bw per day and hematological, renal, and hepatic effects in subchronic and chronic rat studies, using an uncertainty factor of 100. The US EPA has concluded that 2,4-D is unclassifiable with respect to carcinogenicity in humans, based on inadequate data from epidemiological studies and the lack of adequate animal studies.

The WHO (2017) established a guideline value of 0.03 mg/L in 1998, based on an ADI of 0.01 mg/kg bw for the sum of 2,4-D and its salts and esters, expressed as 2,4-D. The ADI was based on a NOAEL of 1 mg/kg of body weight per day established in both a 1-year study of toxicity in dogs (based on serum chemical parameters and histopathological lesions in the liver and kidney) and a 2-year study in rats (based on kidney effects and using an uncertainty factor of 100) (JMPR, 1996; WHO, 2003).

The Australian drinking water guideline of 0.03 mg/L for 2,4-D was first established in 1989 and reconfirmed in 2006. It is based on a no-observed-effect level (NOEL) of 1 mg/kg bw per day for kidney effects in a 2-year rat study, using an uncertainty factor of 100 (NHMRC, NRMMC, 2011).

The European Union (EU) does not have a specific chemical parametric value for individual pesticides. Instead, the EU has a value of 0.1 µg/L for any individual (single) pesticide, and a value of 0.5 µg/L for total pesticides found in drinking water. In establishing these values, the EU did not consider the science related to each pesticide, such as health effects. Instead, the values are based on a policy decision to keep pesticides out of drinking water.

Table 6. Comparison of international drinking water values for 2,4-D

Agency (Year)	**Value** (mg/L)	**Key Endpoint** (Reference)	**NOAEL /NOEL** (mg/kg bw/d)	**UF**	**ADI** (mg/kg bw/d)	**BW** (kg)	**DW Intake** (L/d)	**AF** (%)	**Comments**
HC - proposed MAC (2019)	0.10	Kidney effects in rats and mice (Serota, 1986; Jeffries et al., 1995; Health Canada, 2018b)	5*	300	0.017	74	1.53	20	HBV was calculated as 0.16 mg/L. *The NOAEL for the Serota study (1986) was previously set at 1 mg/kg/d but was re-assessed by a Pathology Working Group in 2005 and increased to 5 mg/kg/d (see Section 2.4)
US EPA (1991)	0.07	Kidney and liver effects in rats (Serota et al., 1983a)	1	100	0.01	70	2	20	Serota et al. (1983a) is the interim report for the Serota (1986) study used as the basis of the HC MAC.
WHO (1998)	0.03	Kidney and liver effects in dogs and kidney effects in rats (JMPR, 1996)	1	100	0.01	60	2	10	JMPR (1996) uses a 1-year dog study (Dalgard, 1993) and a 2-year (unrevised*) rat study (Serota, 1986) as the point of departure.
Australia (2011)	0.03	Kidney effects in rats	1	100	0.01	70	2	10	No reference is provided for the study (NHMRC and NRMMC, 2011).
EU (1998)	0.1 µg/L	The EU has a value of 0.1 µg/L for any individual (single) pesticide, and a value of 0.5 µg/L for total pesticides found in drinking water. In establishing these values, the EU did not consider the science related to each pesticide, including health effects. Instead, the values are based on a policy decision to keep pesticides out of drinking water.							

ADI - Acceptable daily intake
AF - Allocation factor
BD - Body weight
DW - Drinking water
HC - Health Canada
NOAEL - no-observed-adverse-effect level
NOEL - no-observed-effect-level

7.0 Rationale

2,4-D is registered in Canada for use on turf, forests and woodlots, terrestrial feed and food crops, and industrial and domestic non-food sites. It is among the top 10 pesticides sold in Canada. Despite its common use in Canada, exposure data do not indicate significant levels in drinking water (provincial and territorial data show that levels in drinking water are generally below 1 µg/L). Although the IARC classified 2,4-D in group 2B, as possibly carcinogenic to humans (based on limited evidence in animals), international drinking water agencies have assessed 2,4-D based on its non-cancer effects. The kidneys are considered to be the target for 2,4-D's toxicity. Although no studies have investigated the effects of 2,4-D on the kidney in humans, kidney effects have been consistently observed in mice and rat studies. An HBV of 0.16 mg/L has been calculated based on kidney effects in mice and rats.

Health Canada in collaboration with the Federal-Provincial-Territorial Committee on Drinking Water is proposing a MAC of 0.10 mg/L (100 µg/L) for 2,4-D in drinking water based on the following considerations.

- Although an HBV of 0.16 mg/L (160 µg/L) can be calculated, Canadian jurisdictions have shown that a lower proposed MAC of 0.1 mg/L (100 µg/L) is already being consistently achieved.
- Increasing the MAC would provide no reduction in implementation cost and no increase in health protection.
- Analytical methods are available to accurately measure 2,4-D at concentrations well below the proposed MAC.
- Treatment is available to reduce 2,4-D concentrations to the proposed MAC.

The proposed MAC is protective of potential health effects, can be reliably measured by available analytical methods and is achievable by municipal and residential scale treatment technologies. As part of its ongoing guideline review process, Health Canada will continue to monitor new research in this area and recommend any change to this guideline technical document that it deems necessary.

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Appendix A: List of acronyms

2,4-D	2,4-dichlorophenoxyacetic acid
2,4-DCP	2,4-dichlorophenol
ADI	acceptable daily intake
ALP	alkaline phosphatase
ALT	alanine transaminase
ANSI	American National Standards Institute
AOP	advanced oxidation process
APHA	American Public Health Association
AST	aspartate transaminase
AWWA	American Water Works Association
BUN	blood urea nitrogen
BVs	bed volumes
bw	body weight
CAS	chemical abstracts service
CI	confidence interval
DEA	diethanolamine salt
DL	detection limit
DMA	dimethylamine
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
EBCT	empty bed contact time
ECD	electron capture detector
EPA	Environmental Protection Agency (U.S.)
GAC	granular activated carbon
GD	gestation day
GC	gas chromatography
GSH	glutathione
H_2O_2	hydrogen peroxide
HBV	health-based value
IARC	International Agency for Research on Cancer
IPA	isopropylamine
LD_{50}	median lethal dose
LLMED	liquid–liquid microextraction, derivatization
LOD	limit of detection
MAC	maximum acceptable concentration
MDL	method detection limit
NHL	non-Hodgkin's lymphoma
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NOM	natural organic matter
NSF	NSF International
O_3	ozone
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio

PAC	powdered activated carbon
PND	postnatal day
SCC	Standards Council of Canada
STS	soft-tissue sarcoma
T4	thyroxin
UV	ultraviolet
VOCs	volatile organic chemicals
WHO	World Health Organization

Appendix B: Canadian water quality data

Table B1. Levels of 2,4-D and transformation products in Canadian sources from the Environment Canada National Water Quality Surveillance Program (2003–2005).

Jurisdiction (year sampled)	No. detects/ samples	MDL (ng/L)	Range (ng/L)		25th percentile (ng/L)	Mean (ng/L)	Median (ng/L)	75th percentile (ng/L)
			Min	Max				
Tap Water								
AB, SK, MB – rural communities (2004-2005)	?/28		10.50	589.00		81.40		
Surface Water								
BC – Lower Fraser Valley and Okanagan Basin (2003-2005)	59/92	0.5	<0.5	1230	0.680		2.720	22.95
BC – Lower Fraser Valley (2003-2005)			0.62	1230				
BC – Okanagan Basin (2003-2005)			0.59	41.8				
ON (2003)	156/160	0.47	1.3	2850	7.06		46.50	114.50
ON (2004)	184/228	0.47	0.71	8240	1.84		12.75	61.95
ON (2005)	148/183	0.47	0.92	4220	1.59		10.8	76.5
QC (2003)	5/51	20	<20	120				
QC (2004)	25/70	10-20	<10	190				
QC (2004)[b]	23/69	3-50	<3	130				
QC (2005)	13/59	20	<20	340				
NB (2003-2005)	0/57	100						
PEI (2003-2005)	0/82	100						
NS (2003-2005)	0/48	100						
Rivers								
AB, SK, MB – 8 sites (2003)	59/64	0.47	<0.47	457	18.50		46.00	65.00
Reservoir Water								
AB, SK, MB – 15 sites (2003-2004)	205/206	0.47	<0.47	1850	23.85		66.30	129.00
Runoff								
BC – Okanagan Basin (2003-2005)			0.61	445				
Groundwater								
BC – Lower Fraser Valley (2003-2005)				5.01				
BC – Lower Fraser Valley (2003-2005)[a]			0.001	4.93				

MDL = method detection limit
? = Number of detects not given
[a] Represents transformation product desethylatrazine
[b] Represents transformation product deisopropylatrazine
Note: Adapted from Environment Canada, 2011