2,4-D (20)

EXPLANATION

2,4-D was first evaluated in 1970 (T, R), and the last evaluations are from 1986/87 (R) and 1996 (T). The 1997 JMPR evaluated effects on the environment.

2,4-D was proposed for re-evaluation by the Working Group on Priorities at the 1989 CCPR as the ADI was established before 1976 (ALINORM 89/24A, para 299 and Appendix V). It was stated at the 1990 CCPR that there was continued use and manufacturers might be able to submit data (ALINORM 91/24 para 360, Appendix V part I). The review was tentatively scheduled for 1994. The compound was later rescheduled to the 1996 JMPR, and then to the 1998 Meeting.

The present evaluation is within the CCPR Periodic Review Programme.

The members of the industry taskforce which supplied most of the information were AGRO-GOR, Dow Elanco, Nufarm and Rhône Poulenc, who provided data on metabolism and environmental fate, analytical methods, use patterns, residue trials and national MRLs. Information on residue analytical methods, GAP and national MRLs was also provided by the government of The Netherlands.

IDENTITY (Free acid)

ISO common name: 2,4-D
Chemical name

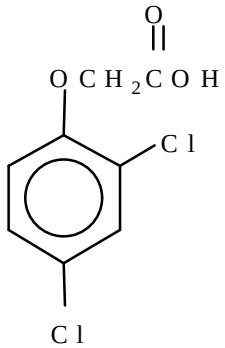
IUPAC: 2,4-dichlorophenoxyacetic acid

CA: (2,4-dichlorophenoxy)acetic acid

CAS Registry No.: 94-75-7

CIPAC No.: 1

Structural formula:



Molecular formula: $C_8H_6Cl_2O_3$

Molecular weight: 221.0

Physical and chemical properties

Pure active ingredient

Appearance: white to brown crystals, granules, flakes, powder or lumps

Vapour pressure: 1.9×10^{-5} Pa at 25°C (Chakrabarti and Gennrich, 1987a)

Melting point: 140.5°C

Octanol/Water partition coefficient at 25°C:
log P_{OW} = 2.7 at pH 1; 0.18 at pH 5; -0.83 at pH 7; -1.01 at pH 9
(Bailey and Hopkins, 1987)

Solubility: in water at 25°C (Hopkins,1987c)

pH 1 buffered	311 ± 4 mg/l
pH 5 buffered	20031± 1149 mg/l
pH 7 buffered	23180 ±590 mg/l
pH 9 buffered	34196 ± 1031 mg/l
pH 5 unbuffered	29934 ± 2957 mg/l
pH 7 unbuffered	44558 ± 674 mg/l
pH 9 unbuffered	43134 ± 336 mg/l

Hydrolysis: stable at pH 5, 7, 9 at 25°C (Anon. 1989a)

Photolysis: aqueous photolysis pH 7 $t_{1/2}$ = 13 days (Anon. 1989b)
soil photolysis very stable

Rate of dissociation: ~200 minutes at 25°C

Dissociation constant: pK_a 3 at 25.2°C (Reim, 1989a; Gallacher, 1991)

Thermal stability: stable at melting point

Technical material

Minimum purity: 96%

Main impurities:

water	1.5% max.
free phenols	0.3% max. (calculated as 2,4-dichlorophenol)
sulphated ash	0.5% max.
triethanolamine insolubles	0.1% max.

Melting range: 137-141°C

Stability: stable indefinitely

Formulations

Commercially available formulations: TC, WP, SP, WG. Also compounded as alkali metal salts, organic amines and esters.

IDENTITY (dimethylamine salt)

ISO common name: 2,4-D-dimethylamine

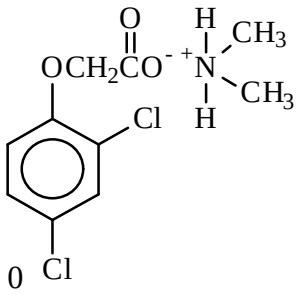
Chemical name
IUPAC: dimethylamine (2,4-dichlorophenoxy)acetate
CA: (2,4-dichlorophenoxy) acetic acid, dimethylamine salt

CAS Registry No.: 2008-39-1

CIPAC No.: 1

Synonyms: 2,4-D DMA

Structural formula:



Molecular formula: $C_{10}H_{13}Cl_2NO_3$

Molecular weight: 266.13

Physical and chemical properties

Pure active ingredient

Appearance: amber to brown liquid; white to brown crystals or powder

Vapour pressure: $<1.33 \times 10^{-5}$ Pa at 26°C (Douglas, 1993a; MacDaniel and Weiler, 1987)

Octanol/Water partition coefficient: refer to 2,4-D acid

Solubility (water: Hopkins, 1987a,b; organic solvents: Kinnunen, 1994a):

water	pH 5	320632 ± 3645 mg/l at 25°C
	pH 7	729397 ± 86400 mg/l at 25°C
	pH 9	663755 ± 94647 mg/l at 25°C
acetonitrile		1.02 g/100 ml at 20°C
methanol		>50 g/100 ml at 20°C
hexane		3.59 g/100 ml at 20°C
1-octanol		5.37 g/100 ml at 20°C
toluene		0.165 g/100 ml at 20°C

Specific gravity: 1.23 - 1.24 g/cm^3 at 20°C (Dow Chemical Co., 1989a)

Hydrolysis: refer to 2,4-D acid

Photolysis: refer to 2,4-D acid

Rate of dissociation: <1 minutes

Thermal stability: stable

pH: 6.8 -9

Technical material

Minimum purity: to be prepared from 2,4-D acid 96% min.

Main impurities: proportional to 2,4-D content except water

Melting range: 118–120°C ± 1°C (Murphy, 1993a)

Formulations

Commercially available formulations: TK, SL, SP.

IDENTITY (2-ethylhexyl ester)

ISO common name: 2,4-D-ethylhexyl

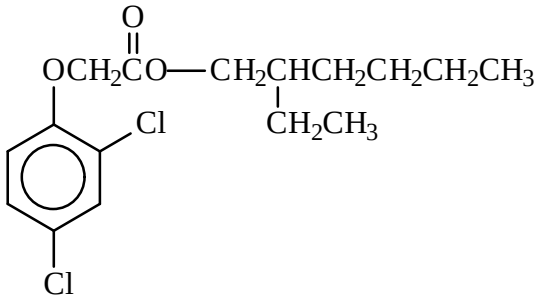
Chemical name
IUPAC: 2-ethylhexyl (2,4-dichlorophenoxy)acetate
CA: (2,4-dichlorophenoxy) acetic acid, 2-ethylhexyl ester

CAS Registry No.: 1928-43-4

CIPAC No.: 1

Synonyms: 2,4-D EHE

0
Structural formula:



Molecular formula: $C_{16}H_{22}Cl_2O_3$

Molecular weight: 333.27

Physical and chemical properties

Pure active ingredient

Appearance: amber to brown liquid

Vapour pressure: 4.8×10^{-4} Pa at 25°C (Chakrabarti and Gennrich, 1987b)

Boiling point: >200°C under degradation (Kinnunen, 1994b)

Octanol/Water partition coefficient: log P_{OW} = 5.78 at 25°C (Helmer, 1987a)

Solubility: water 0.0867 mg/l at 25°C (Helmer, 1987b)
industrial water 0.0324 ± 0.0032 mg/l at 12°C (Potter, 1990)

Specific gravity: 1.152 g/cm^3 at 20°C (Dow Chemical Co., 1989b)

Hydrolysis: in sterile aqueous solutions at 25°C (Concha *et al.*, 1993a):
pH 5 $t_{1/2}$ 99.7 days
pH 7 $t_{1/2}$ 48.3 days
pH 9 $t_{1/2}$ 52.2 hours
in natural water (river water) pH 7.8 at 25°C:
$t_{1/2}$ 6.2 hours
in soil slurries at 25°C (Concha *et al.*, 1993b):
$t_{1/2}$ in Catlin silty clay soil slurry 1.25 hours
$t_{1/2}$ in Hanford sandy loam soil slurry 1.45 hours

Photolysis: sterile aqueous photolysis 25°C $t_{1/2}$ 128 days (Concha and Shepler, 1993b)
UV stable (Schriber and Tiszai, 1991)

Technical material

Minimum purity: 92%

Main impurities: proportional to 2,4-D content except:
free acid 1.5% max., suspended solids 0.1% max., water 1% max.

Stability: no appreciable change in 2 years in sealed containers. will decompose before boiling.

Formulations

Commercially available formulations: TK, EC, EW and OL.

IDENTITY (diethanolamine salt)

ISO common name: 2,4-D-diethanolamine

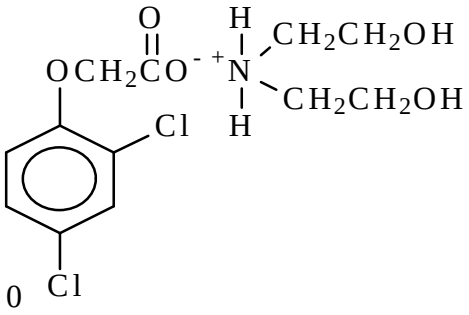
Chemical name
IUPAC: diethanolamine (2,4-dichlorophenoxy)acetate
CA: (2,4-dichlorophenoxy) acetic acid, diethanolamine salt

CAS Registry No.: 5742-19-8

CIPAC No.: 1

Synonyms: 2,4-D DEA

Structural formula:



Molecular formula: $C_{12}H_{17}Cl_2NO_5$
Molecular weight: 326.18

Physical and chemical properties

Pure active ingredient

Appearance: cream solid powder

Vapour pressure: $<1.33 \times 10^{-5}$ Pa at 25°C and 45°C (Douglas, 1993a)

Melting point: 83°C (Malone, 1993)

Octanol/Water partition coefficient: log P_{OW} = -1.65 at 25°C (Douglas, 1993b)

Solubility:		
	acetonitrile	47 mg/g at 25°C
	ethanol	280 mg/g at 25°C
	n-octanol	36 mg/g at 25°C
	water	806 mg/g at 25°C (Douglas, 1993c)

Specific gravity: 0.762 g/cm^3 at 25.5°C (Wojcieck, 1992a)

Photolysis: stable under light

Rate of dissociation: 3 minutes at 25°C

Thermal stability: stable up to 150°C (Malone, 1993)

pH: 7.48 at 25°C (Furlong, 1992)

Technical material

No information was received.

FORMULATIONS

Commercially available formulations: SL.

IDENTITY (2-butoxyethyl ester)

ISO common name: 2,4-D-butoxyethyl

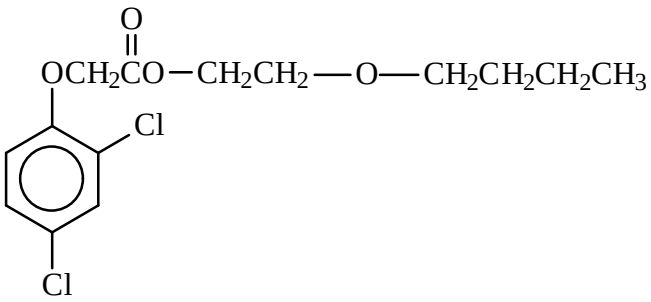
Chemical name
IUPAC: 2-butoxyethyl (2,4-dichlorophenoxy)acetate
CA: (2,4-dichlorophenoxy) acetic acid, 2-butoxyethyl ester

CAS Registry No.: 1929-73-3

CIPAC No.: 1

Synonyms: 2,4-D BEE

Structural formula:



Molecular formula: $C_{14}H_{18}Cl_2O_4$

Molecular weight: 321.20

Physical and chemical properties

Pure active ingredient

Appearance: amber liquid

Vapour pressure: 3.2×10^{-4} Pa at 25°C (Chakrabarti, 1989)

Boiling point: 89°C (Kinnunen, 1994c)

Octanol/Water partition coefficient: log P_{OW} = 4.1 at 25°C (Heimerl, 1990)

Specific gravity: 1.0 - 1.2 g/ml at 20°C (Dow Chemical Co., 1989c)

Hydrolysis: in sterile water buffered at 25°C (Shepler *et al.*, 1990)
pH5 $t_{1/2}$ 196 days
pH7 $t_{1/2}$ 74 days
pH9 $t_{1/2}$ 55 minutes
soil/water slurry (Racke, 1989)
61% hydrolysed in 20 minutes

Photolysis: (aqueous) $t_{1/2}$ 74 days, stable to photodegradation (Marx and Shepler, 1990)

Thermal stability: stable up to 50°C (Schriber, 1992)

Technical material

Minimum purity: 92.0%

Stability: stable for a minimum of 2 years in sealed container.

Formulations

Commercially available formulations: EC, TK.

IDENTITY (isopropylamine salt)

ISO common name: 2,4-D-isopropylamine

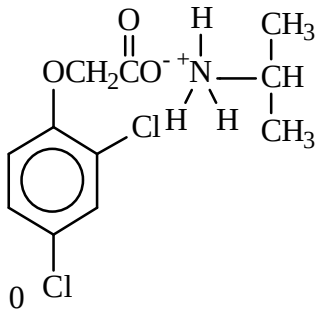
Chemical name
IUPAC: isopropylamine (2,4-dichlorophenoxy)acetate
CA: (2,4-dichlorophenoxy) acetic acid, isopropylamine salt

CAS Registry No.: 5742-17-6

CIPAC No.: 1

Synonyms: 2,4-D IPA

Structural formula:



Molecular formula: $C_{11}H_{15}Cl_2NO_3$

Molecular weight: 280.04

Physical and chemical properties

Pure active ingredient

Appearance: amber liquid

Vapour pressure: <1.33 x 10^{-5} Pa (Chakrabarti, 1990a)
(salt decomposed in temperature range -3.9 to 24°C)

Octanol/Water partition coefficient: refer to 2,4-D acid

Melting point: 121°C ± 1°C (Murphy, 1993b)

Solubility (Kinnunen, 1994d):

water	pH5	17.4 g/100 ml
	pH7	43.6 g/100 ml
	pH9	33.1 g/100 ml
acetonitrile		2.1 g/100 ml

	methanol	>50 g/100 ml
	hexane	4.36×10^{-3} g/100 ml

Specific gravity: 1.15 g/ml at 20°C (Dow Chemical Co., 1989d)

Photolysis: refer to 2,4-D acid

Dissociation rate: <1 min at 21°C (Reim, 1989b)

Thermal stability: stable up to 50°C (Schriber, 1991a)

Technical material

Minimum purity: 48.4%

Stability: stable for minimum of 2 years in sealed container

Formulations

Commercially available formulations: SL.

IDENTITY (isopropylamine salt)

ISO common name: 2,4-D-isopropylamine

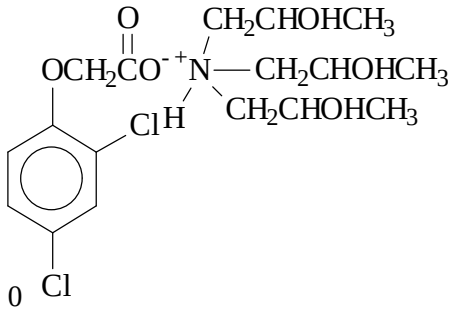
Chemical name
IUPAC: tri-isopropanolamine (2,4-dichlorophenoxy)acetate
CA: (2,4-dichlorophenoxy) acetic acid, tri-isopropanolamine salt

CAS Registry No.: 32341-80-3

CIPAC No.: 1

Synonyms: 2,4-D TIPA

Structural formula:



Molecular formula: $C_{17}H_{27}Cl_2NO_6$

Molecular weight: 412.31

Physical and chemical properties

Pure active ingredient

Appearance: amber liquid

Vapour pressure: <1.33 x 10^{-5} Pa at 14.2 - 28.0°C (Chakrabarti, 1990b)

Octanol/Water partition coefficient: refer to 2,4-D acid

Melting point: 88.0°C - 110.5°C (Kinnunen, 1994)

Solubility (Schriber, 1991b):

water	pH5	46.1 wt % ai
	pH7	46.1 wt % ai
	pH9	10.4 wt % ai
acetonitrile		12.3 wt % ai
acetone		11.7 wt % ai
n-octanol		7.6 wt % ai

Specific gravity: 1.2 g/ml at 20°C (Dow Chemical Co., 1989e)

Photolysis: refer to 2,4-D acid

Dissociation rate: <1 min at 21°C (Reim, 1989b)

Thermal stability: stable up to 50°C (Schriber, 1991c)

Technical material

Minimum purity: 68.8%

Melting range: 88°C - 110.5°C (Kinnunen, 1994e)

Stability: stable at minimum for 2 years in sealed container

Formulations

Commercially available formulations: SL.

IDENTITY (isopropyl ester)

ISO common name: isopropyl

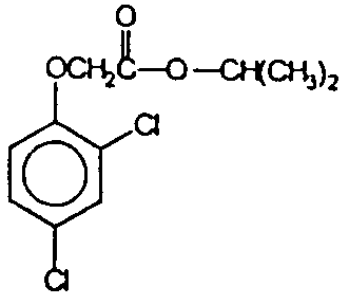
Chemical name
IUPAC: (2,4-dichlorophenoxy)acetate, isopropyl ester
CA: (2,4-dichlorophenoxy) acetic acid, isopropyl ester

CAS Registry No.: 94-11-1

CIPAC No.: 1

Synonyms: 2,4-D IPE

Structural formula:



Molecular formula: $C_{11}H_{12}Cl_2O_3$

Molecular weight: 263.12

Physical and chemical properties

Pure active ingredient

Appearance: light amber liquid

Vapour pressure: 1.87 Pa at 25°C (Fisher, 1989)

Octanol/Water partition coefficient: log P_{ow} = 4.2 at 25°C (Fisher, 1989)

Solubility: practically insoluble in water (Fisher, 1989)

Specific gravity: 1.25 g/cm^3 at 20°C (Fisher, 1989)

Hydrolysis: under sterile conditions at 25°C

pH 5	no hydrolysis in 30 days	
pH 7	$t_{1/2}$	89 days
pH 9	$t_{1/2}$	22.4 hours (Burke, 1994a)

Thermal stability: stable to approximately 240°C (Fisher, 1989)

Technical material

Minimum purity: 92%

Main impurities: proportional to 2,4-D content (except free acid 1,5% max. suspended solids 0.1% max., water 1% max.)

Stability: no appreciable change in 2 years in sealed containers. Decomposes before boiling.

Formulations

Commercially available formulations: TK and EC.

METABOLISM AND ENVIRONMENTAL FATE

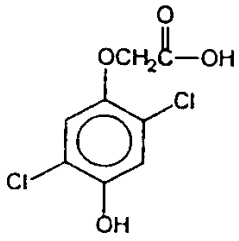
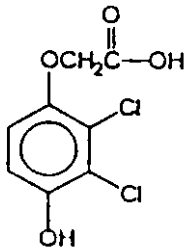
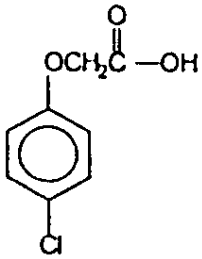
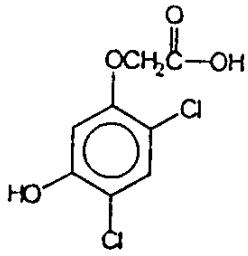
Metabolism and environmental fate studies were conducted with uniformly ring-^{14}C-labelled 2,4-D:

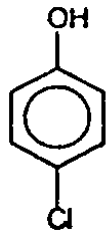
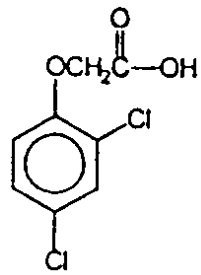
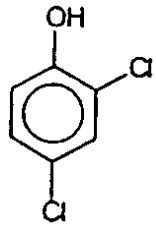
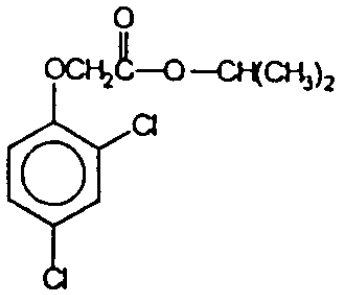
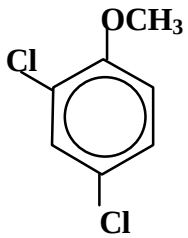
^{14}C-labelled 2,4-dichlorophenoxyacetic acid ([^{14}C]2,4-D): mouse, rat, goat, poultry, fish.
^{14}C-labelled 2,4-dichlorophenoxyacetic acid dimethylamine salt ([^{14}C]2,4-D DMA): apple.
^{14}C-labelled 2,4-dichlorophenoxyacetic acid 2-ethylhexyl ester ([^{14}C]2,4-D EHE): potato, wheat.
^{14}C-labelled 2,4-dichlorophenoxyacetic acid isopropylester ([^{14}C]2,4-D IPE): lemon.

Furthermore, the label on the ester moiety is also used:
2,4-Dichlorophenoxyacetic acid 2-ethylhexyl-1-^{14}C ester (2,4-D EHE-1-^{14}C): rat.

For the chemical names, structures and abbreviations of metabolites used in the text see Table 1.

Table 1. Structures of metabolites of 2,4-D.

Chemical name, abbreviation	Structure
2,5-Dichloro-4-OH-phenoxyacetic acid (4-Hydroxy-2,5-D)	
2,3-Dichloro-4-OH-phenoxyacetic acid (4-Hydroxy-2,3-D)	
4-Chlorophenoxyacetic acid (4-CPAA)	
2,4-Dichloro-5-OH-phenoxyacetic acid (5-Hydroxy-2,4-D)	

Chemical name, abbreviation	Structure
4-Chlorophenol (4-CP)	
2, 4-Dichlorophenoxyacetic acid (2,4-D)	
2,4-Dichlorophenol (2,4-DCP)	
2,4-Dichlorophenoxyacetic acid isopropyl ester (2,4-D IPE)	
2,4-Dichloroanisole (2,4-DCA)	

Animal metabolism

Mice (Eiseman, 1984). The pharmacokinetics of [^{14}C]2,4-D (purity, 98%) were studied in groups of 26 male B6C3F1 mice after single oral doses at 5, 45, or 90 mg/kg bw and single intravenous

administrations of 90 mg/kg bw. To evaluate the excretion balance groups of five mice were given the same single doses of [^{14}C]2,4-D by gavage or intravenous doses of 5 or 90-mg/kg bw. Plasma, liver and kidneys were analysed for the radiolabel 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 hours after treatment. Urine was collected before and after treatment at 0-6, 6-12, 12-24, 24-36, 36-48, 48-72 hours and then every 24 hours up to 168 hours. Faeces were collected before and 0-12, 12-24 and every 24 hours up to 168 hours after treatment. The animals were then slaughtered and the blood, liver, kidneys and residual carcases were sampled for measurement of total radioactive residues (TRR).

The disappearance of the label from plasma in animals at each dose was examined by reiteratively weighted non-linear regression analysis to obtain the apparent pharmacokinetic parameters by both the oral and intravenous routes. Because of the apparent lag in clearance at the higher doses and the high levels of 2,4-D-derived ^{14}C in plasma during the first 4 hours after dosing, a two-compartment model with Michaelis-Menten limited clearance was used. The half-lives were calculated to be 28-45 hours. At least 50% of the dose was cleared within 12 hours however, suggesting that the estimates are lower than the actual clearance constants.

After oral administration, the area under the curve (AUC) of time v. concentration increased more than proportionally with the dose.

The main route of elimination of ^{14}C was the urine, accounting for 63, 84, 71, 53 and 65% of doses at 5 mg/kg bw orally, 5 mg/kg intravenously, 45 mg/kg bw orally, 90 mg/kg bw orally, and 90 mg/kg intravenously respectively. 7.6% of the dose in animals receiving 5 mg/kg bw orally and 5.2% at 5 mg/kg bw intravenously was excreted in the faeces. The proportions increased to 15% at 45 mg/kg bw orally, 16% at 90 mg/kg bw orally, and 12% at 90 mg/kg bw intravenously. Most of the TRR was eliminated in the urine 0-6 hours after treatment at 5 mg/kg bw intravenously, 0-12 hours after at 5 mg/kg bw orally, and 6-24 hours after at 45 or 90 mg/kg bw. 168 hours after treatment very little ^{14}C was detected. None was found in blood or plasma in the animals dosed intravenously, and only one animal of those dosed orally had a detectable, low level of radiolabel in the plasma. The liver and kidneys contained similar levels of TRR at each dose. Less than 1.1% of the dose was retained in the animals seven days after administration of [^{14}C]2,4-D, irrespective of the dose and administration route. The urinary clearance of 2,4-D appeared to be a saturable process in male mice at doses of $\geq$45 mg/kg bw.

Rats. Smith *et al.* (1980) examined the pharmacokinetics of the elimination of [^{14}C]2,4-D (radiochemical purity, >99%) after oral and intravenous administration to several groups of male Fischer 344 rats to investigate the fate of the compound as a function of the dose and to identify the approximate dose at which the kinetics of elimination begin to show evidence of saturation. Three groups of three rats with jugular cannulae received oral doses of 10, 50, or 150 mg/kg bw, and two similar groups received intravenous doses of 5 or 90 mg/kg bw. The concentrations of TRR were determined 1, 2, 3, 6, 9, 12,15, 18, 24, 36, 48, 60 and 72 hours after treatment; ^{14}C levels in the urine were measured at 6-hourly intervals for the first 24 hours and at 12-hour intervals thereafter up to 72 hours; faecal samples were collected at 24-hour intervals.

To determine the effect of dose five groups of six rats were given single oral doses of 10, 25, 50, 100 or 150 mg/kg bw and were killed 6 hours after treatment. Absorption of 2,4-D after oral administration was complete as >85% of the dose within the first 12 hours and a total of 97% of the 10 mg/kg bw oral dose and 95% of the 150 mg/kg bw dose was excreted in the urine. After intravenous doses 99 and 86% of the 5- and 90-mg/kg bw doses were recovered within the first 12 hours and 100 and 91% after 72 hours. Saturable clearance from the plasma was detected and was confirmed by the disproportionate increase in the AUC, which probably reflects saturable urinary excretion, in view of the concomitant increasing ratio of plasma:kidney ^{14}C concentrations with increasing dose.

The elimination was biphasic. The mean half-lives for the intravenous and oral routes were 55 minutes and 1 hour respectively for the ∀-phase and 14 and 18 hours for the ∃-phase. The rapid elimination of the ^{14}C in the urine and the small contribution of the ∃-phase indicate low potential accumulation of 2,4-D in rats.

Timchalk (1990) examined the absorption, distribution, metabolism and excretion of [^{14}C]2,4-D after oral and intravenous administration to Fischer 344 rats. Four groups of five male and five female rats each received [^{14}C]2,4-D as a single oral dose by gavage at 1 or 100 mg/kg bw, or as a single intravenous dose at 1 mg/kg bw, or were given 14 daily oral doses of unlabelled 2,4-D at 1 mg/kg bw followed by a single oral dose of [^{14}C]2,4-D at the same rate on day 15. Two additional groups of four male rats were given single oral doses and then 1 or 100 mg/kg bw through jugular cannulae to define the concentration-time course in the plasma. Plasma concentrations were determined for 24 hours after treatment.

In all groups >94% of the dose was recovered within 48 hours after treatment, mainly in the urine (85-94%), with 2-11% in the faeces. No sex-related difference was seen, and repeated oral dosage did not alter the excretory route. Peak plasma levels were attained about 4 hours after treatment. The disproportionate AUCs and the delayed urinary excretion of ^{14}C strongly imply dose-dependent non-linear kinetics however. Although the elimination of the radiolabel was saturated during the first few hours after the 100 mg/kg bw dose its excretion was rapid, most of the dose having been excreted 36 hours after treatment in all groups. Rapid excretion of [^{14}C]2,4-D is also indicated by the approximate half-life of 5 hours for urinary excretion after oral administration. The analysis of all major tissues and organs for residual ^{14}C activity indicated that only a small fraction of the dose was present 48 hours after treatment. The tissues and organs of animals treated at the low dose contained <0.7% of the administered [^{14}C]. These results indicate that the fate of [^{14}C]2,4-D in rats is independent of dose and sex and that the compound is rapidly and almost completely eliminated, essentially by the urinary route, and is unlikely to accumulate.

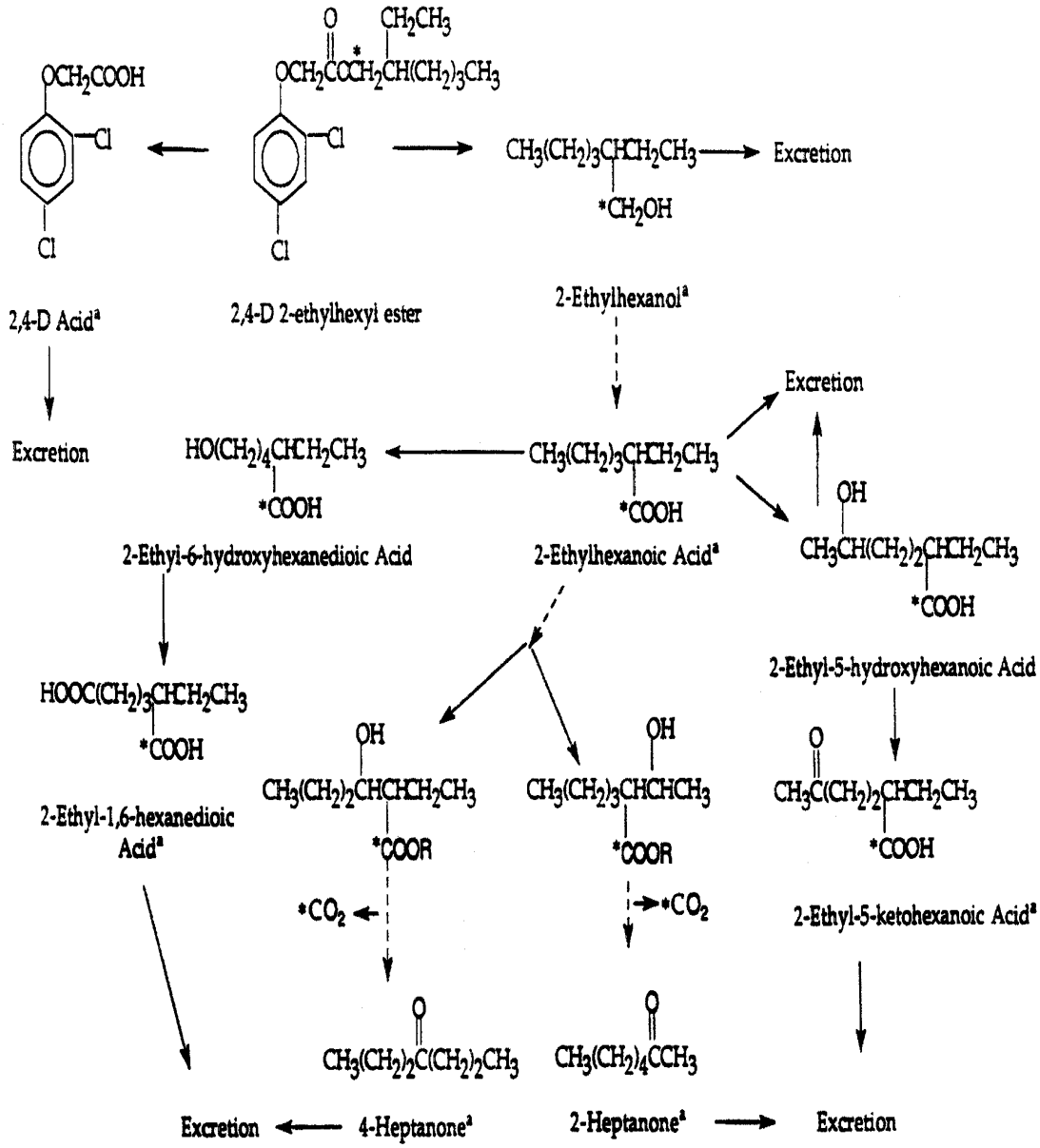
Unlabelled 2,4-D EHE was administered as a single oral dose to male and female 11 week old Fischer 344 rats to investigate the pharmacokinetic characteristics of this ester in relation to 2,4-D itself (Frantz and Kropscott, 1984). Eight groups of animals (3/sex/group) were given oral doses of 130 mg/kg of the 2-ethylhexyl ester (equivalent to 86.3 mg/kg 2,4-D acid) in maize oil and killed after 15 and 30 min, and 1, 2, 4, 8, 24 and 72 hours for blood samples. Urine samples were taken at 12 hour intervals from the 72-hour group. A control group (3/sex) was dosed with maize oil only and a terminal blood sample was taken after 3 hours. Both ester and acid were determined by GLC and GC-MS with detection limits of 0.01 and 0.1 :g/ml respectively.

The most significant finding was the absence of any 2-ethylhexyl ester ([0.01 :g/ml) in either blood or urine as measured up to 72 hours after dosing. The acid was found in both blood and urine. Log-linear plots of 2,4-D acid concentration in the blood v. time were similar for male and female rats, with peak blood levels appearing at 2 hours for females and 4 hours for males. No acid was detectable at 72 hours in either male or female blood samples. Urine levels of 2,4-D acid peaked at 12 hours in both sexes and clearance was nearly complete by 36 hours; only small amounts of acid were detectable at 72 hours. The cumulative recovery of 2,4-D acid in urine (as a percentage of the equivalent administered) was 94.8 ± 9.2% for males and 84.3 ± 4.5% for females. These data indicate that the 2-ethylhexyl ester of 2,4-D is converted very rapidly to 2,4-D acid, and the acid is then excreted in the urine.

The absorption, distribution, excretion and biotransformation of [*EHE*-1-^{14}C]2,4-D were studied in male Fischer 344 rats after a single oral dose of 15 mg/kg bw (Dryzga *et al.*, 1992). Blood was collected from each rat 0.5, 1, 1.5, 2, 4, 6, 8, 12, 18 and 24 hours after treatment and the plasma was analysed for the radiolabel; urine was collected 6, 12, 24 and 48 hours after treatment, and the radiolabel in the urine and the cage rinse was combined for each collection interval and expressed as radiolabel excreted in the urine. Faeces were collected at 24-hour intervals and analysed for

radiolabel; expired [^{14}C]carbon dioxide collected at 6, 12, 24 and 48 hours was trapped in a solution of monoethanolamine and 1-methoxy-2-propanol, and the TRR was quantified. No tissue samples were analysed. Metabolites were characterized in pooled urine (0-6 and 6-12 hours) and in faeces (0-24 hours) by GC-MS, and unchanged ester in the urine and faecal extracts was determined by HPLC.

Figure 1. Proposed metabolic pathways of 2,4-Dd EHE in rats (Dryzga *et al.*, 1992).



[a] Compounds actually identified

,4-D EHE-1-^{14}C was rapidly absorbed, with a peak plasma concentration of 1.0 mg/g 4 h after treatment, decreasing with a half-life of 9 hours. Once absorbed, the ester was extensively metabolized and eliminated in the urine and faeces, and as expired $^{14}CO_2$. It was rapidly hydrolysed to 2,4-D and 2-ethylhexanol, since no ester was found in the blood, urine, or faeces. The principal route of excretion was the urine (62-66%), with less in the faeces (14-21%) and expired carbon dioxide (9-12%). The metabolites in both urine and faeces were 2-ethylhexanol, 2-ethylhexanoic

acid, 2-ethylhexane-1,6-dioic acid and 2,4-D. Metabolites found in only in the urine were 2-ethyl-5-oxohexanoic acid, 2-ethyl-5-hydroxyhexanoic acid, 2-heptanone and 4-heptanone (Figure 1). These metabolites were previously reported as metabolites of [^{14}C]2-ethylhexanol. The ethylhexyl ester of 2,4-D is thus converted rapidly to 2,4-D, which is then excreted in the urine.

Goats. Guo and Stewart (1993) dosed a lactating goat orally with [^{14}C]2,4-D at a level equivalent to 483 ppm in the feed intake, for 3 consecutive days. Urine, faeces and milk were sampled during the dosing phase and tissues were collected after slaughter. ^{14}C was determined by combustion and/or liquid scintillation counting. Approximately 82 and 8% of the total dose was recovered in the urine and faeces respectively. Less than 0.1% of the dose was recovered in the other samples.

The ^{14}C residues in the samples except excreta and liver were extracted with organic and aqueous solvents. Liver was first treated with pancreatin and then extracted with organic solvents. Urine was analysed directly by HPLC with radiometric detection. HPLC was used to determine the total ^{14}C as 2,4-D equivalents in the various extracts. The major ^{14}C component was identified as 2,4-D by mass spectrometry, HPLC and thin-layer chromatography. The results of these analyses are shown in Table 2.

Table 2. Metabolism of 2,4-D in goats. Identity and distribution of ^{14}C components in the extractable fractions urine, milk and tissues (Guo and Stewart, 1993).

Sample	Total ^{14}C mg/kg	% 2,4-D	% DCP[1]	% CPAA[2]	% NP1	% NP2[3]	% NP3	% Not[4] identified
Urine	320	97.8	ND[5]	1.8	ND[5]	ND[5]	ND[5]	0.4
Milk	0.202	47.0	5.0	6.9	1.0	ND[5]	ND[5]	35.1
Liver	0.224	20.5	ND[5]	ND[5]	14.7	17.9	5.4	36.6
Kidney	1.44	53.6	ND[5]	ND[5]	10.3	22.0	4.1	9.9
Fat	0.088	45.4	2.3	ND[5]	3.4	13.6	ND[5]	21.7
Muscle	0.037	37.8	ND[5]	ND[5]	2.7	24.3	ND[5]	19.0

[1]DCP: 2,4-dichlorophenol
[2]CPAA: 2-*o* and 2-*p*-chlorophenoxyacetic acid
[3]The GC/MS profile for NP2 matched 2,4-dichloroanisole, but the compound was not later confirmed (Guo and Stewart 1994)
[4]Total % extracted - Total % identified. In general, this radioactivity is associated with several different fractions and with radioactivity that was unaccounted for after HPLC analysis. Thus, no single unidentified component is present at the level shown here.
[5]Not detected

2,4-dichlorophenoxyacetic acid was found to be the predominant component in the urine, kidneys, fat, liver and muscle. Free 2,4-D and polar conjugates hydrolysed to 2,4-D under acidic conditions were the major ^{14}C components in the milk. Low levels of DCP were tentatively identified in the milk and fat by HPLC.

The ^{14}C in the am and pm milk samples ranged from 0.22 to 0.34 mg/kg and 0.036 to 0.055 mg/kg, as 2,4-D respectively. Free [^{14}C]2,4-D was identified as the most significant residue in milk (38% of the TRR, 0.077 mg/kg). Certain polar conjugates were observed in the milk, from which 2,4-D was readily released by acid hydrolysis. The total 2,4-D after a 1-hour hydrolysis was about 47.0% of the TRR or 0.095 mg/kg. Approximately 0.009 mg/kg (4.5% of the TRR) and 0.01 mg/kg (5.0% of the TRR) of the residues in milk were tentatively identified as *o*- and *p*-

chlorophenoxyacetic acid (CPA) and 2,4-dichlorophenol (DCP) respectively. It is possible that CPA derived from an impurity in the original test substance.

The highest ^{14}C concentration in the tissues was in the kidneys (1.4 mg/kg). Lower levels were found in the liver (0.22 mg/kg), fat (0.088 mg/kg) and muscle (0.037 mg/kg). The main component identified in the residues of each tissue was 2,4-D. About 0.002 mg/kg of DCP (2.3% of the TRR) was found in the fat.

Poultry. Residues in the tissues, eggs and excreta were measured in laying hens (3 groups of 5, each bird weighting 1.5 kg) dosed orally for 7 days with radiolabelled capsules of 2,4-D approximately equivalent to 18 ppm in the feed intake (112-119 g/bird/day). The eggs and excreta were collected throughout the 7 days, and the birds were killed 22-24 hours after the final dose (Puvanesarajah and Bliss, 1992).

Samples collected included excreta, egg, fat, gizzard, heart, kidney, liver, breast muscle and thigh muscle. Except for the eggs (production of which from each treatment group was good) all the samples were composited for each group before analysis. The weights of the comparable samples from the three groups were very similar.

The TRR levels in the samples were determined either by direct radio-analysis or combustion and radio analysis (the latter corrected for sample and oxidizer recoveries). Recoveries in the hens ranged from 95.8 to 101.6%, approximately 90% in the excreta. The residues in the breast muscle were <0.002 mg/kg; thigh muscle 0.006 mg/kg; fat 0.028 mg/kg and liver 0.03 mg/kg. The mean residue levels in eggs increased steadily from less than quantifiable at day 1 to 0.018 mg/kg at day 7. The tissues and eggs contained <0.1% of the total dose (Table 3).

Table 3. 2,4-D acid equivalents and % total dose recoveries in body tissues, eggs and excreta of hens (Puvanesarajah and Bliss, 1992).

Sample	Group II		Group III		Group IV	
	TRR, mg/kg[1]	% Total dose recovered	TRR, mg/kg[1]	% Total dose recovered	TRR, mg/kg[1]	% Total dose recovered
Egg 1[2]	<LOD[4]	<0.1	<MQL[4]	<0.1	<MQL[4]	<0.1
Egg 2	0.002	<0.1	0.003	<0.1	0.003	<0.1
Egg 3	0.006	<0.1	0.006	<0.1	0.006	<0.1
Egg 4	0.010	<0.1	0.010	<0.1	0.010	<0.1
Egg 5	0.014	<0.1	0.013	<0.1	0.009	<0.1
Egg 6	0.016	<0.1	0.016	<0.1	0.018	<0.1
Egg 7[3]	0.018	<0.1	0.017	<0.1	0.019	<0.1
Fat	0.029	<0.1	0.032	<0.1	0.023	<0.1
Kidney	0.705	<0.1	0.065	<0.1	0.791	<0.1
Liver	0.025	<0.1	0.019	<0.1	0.046	<0.1
Breast muscle	<MQL[4]	<0.1	<MQL[4]	<0.1	0.002	<0.1
Thigh muscle	0.005	<0.1	0.004	<0.1	0.008	<0.1
Heart	0.011	<0.1	0.008	<0.1	0.028	<0.1
Gizzard	0.142	<0.1	0.038	<0.1	0.118	<0.1
Excreta[5]	16 - 21	95.9	15 - 19	89.9	15 - 21	89.4
Total dose recovered		96.0		90.0		89.5

[1] Numbers listed are the means of triplicate determinations of pooled samples.
[2] First day of dose
[3] Last day of dose
[4] Limit of determination (0.002 mg/kg)
[5] Range of values for the day 1-7 samples

The ^{14}C components present as free residues in the eggs and liver were 2,4-D and 2,4-dichlorophenol (Table 4). A large proportion of the fat activity was released only after base hydrolysis, which suggested that 2,4-D conjugates constituted most of the extractable ^{14}C.

Table 4. Components of extractable ^{14}C residues in hens (Puvanesarajah and Bliss, 1992).

Sample	Total ^{14}C mg/kg	% of extractable ^{14}C (free and conjugated)		
		2,4-D	2,4-Dichlorophenol	Not identified
Egg	0.0178	23.0	7.3	56.8
Fat	0.0271	25.1		67.6
Liver	0.0297	18.2	4.4	59.7

Fish. Bluegill sunfish (*lepomis macrochirus*) were exposed to approximately 11 mg/kg of [^{14}C]2,4-D in their water under static conditions for 4 consecutive days (Premkumar and Stewart, 1994). The whole fish and the water were sampled daily and the fish on day 4 were dissected into fillet and viscera. The [^{14}C]2,4-D equivalent TRR levels in the whole fish, fillet and viscera and the water were determined by combustion and/or liquid scintillation counting.

The fillet and viscera residues were extracted with polar and nonpolar organic solvents. Most of the residues were acetonitrile-extractable. <5% of the TRR was hexane-soluble, and approximately 10% was unextractable with solvents. Acid and base hydrolyses released the unextractable residues in the fillets.

Radio analyses of the whole fish showed a steady increase from day 1 (0.41 mg/kg) to day 3 (0.6 mg/kg). The TRR in the day 4 viscera and fillet were 1.9 and 0.41 mg/kg respectively. The treated fillet had small amounts of hexane-soluble residues (0.005 mg/kg, 1.2% of the TRR) and unextractable ^{14}C (0.041 mg/kg, 10% of the TRR). Most of the ^{14}C was soluble in acetonitrile (0.34 mg/kg, 84% of the TRR).

HPLC radio-analysis was used to characterize the ^{14}C residues in the extracts (Table 5). 2,4-D (70.4% of the TRR, 0.29 mg/kg), 2,4-DCP (4.7% of the TRR, 0.019 mg/kg), and a conjugate (4.4% of the TRR, 0.018 mg/kg) were identified in the acetonitrile-soluble residue. Acid hydrolysis of the polar conjugate released 2,4-D and 2,4-DCP. Acid and base hydrolyses of the unextractable fillet pellet released additional 2,4-D (6.4 and 3.9% of the TRR, 0.029 and 0.016 mg/kg respectively) and 2,4-DCP (0.5% of the TRR, 0.002 mg/kg). The identities of 2,4-D and 2,4-DCP in the fillet tissue were confirmed by GC-MS.

Table 5. Components of extractable ^{14}C residues in fish exposed for 4 days to 11 mg/kg in their water (Premkumar and Stewart, 1994).

TRR		Extractable ^{14}C in					
		2,4-D	2,4-DCP	2,4-DCA	polar unknown	CPA and CP	not identified
Fillet, TRR	% of TRR[1]	80[2]	7.9[2]		2.2	N/A[3]	1.0
0.41 mg/kg	mg/kg[1,2]	0.33	0.03		0.009	N/A[3]	0.004
Viscera, TRR	% of TRR[1]	30[2]	28[2]	0.3[3]	5.8	40	1.6
1.9 mg/kg	mg/kg[1,2]	0.57	0.53	0.006	0.11	0.76	0.03

[1] Sum of free and conjugated
[2] Expressed as 2,4-D
[3] Identity confirmed by GC-MS

Plant metabolism

Apples. Smith (1991) sprayed [^{14}C]2,4-D on the turf beneath the canopy of a dwarf apple tree in accordance with label instructions. The test material consisted of the dimethylamine salt of 2,4-D acid in aqueous solution applied at 2.2 kg ae/ha (2 applications 42 days apart). Soil samples were taken after 42 days and 97 days, and fruit samples after 97 days (maturity) .

Combustion analysis and liquid scintillation counting were used to determine ^{14}C residues in the soil and plant samples. All residues were corrected for oxidizer recovery and matrix effects, and those in the soil for moisture content. Most residues were in the 0-7.5 and 7.5-15 cm layers with considerably less (<0.05 mg/kg as 2,4-D) in the 15-22.5, 22.5-30 and 30-45 cm layers. The TRR in was 0.53 mg/kg and 0.042 mg/kg 0-7.5 and 7.5-15 cm layers at day 42 and 0.93 mg/kg and 0.15 mg/kg at day 97. Combustion analysis of the apples gave TRR levels of 0.009 mg/kg. The apples were lyophilized and exhaustively extracted with methanol/acetic acid (95:5). The extract was then concentrated, re-suspended in water, and extracted with hexane/diethyl ether/acetic acid (47.5:47.5:5), leaving 56% as water-soluble residue with no quantifiable residue (<0.0008 mg/kg) in the organic extract and 44% remaining in the post-extraction solids as determined by combustion.

Lemons. Wu (1994) treated lemons with an aqueous emulsion of [^{14}C]2,4-D isopropyl ester resulting in an average residue level of 2.4 mg/kg expressed as 2,4-D on the fruit. The lemons were stored inside a constant temperature incubator at an average temperature of 5.5 ± 0.7°C, with relative humidity of about 85%. The stored lemons were sampled at 2 hours, 2, 7, 14 and 28 days, and 6, 8, 10, 12, 16, 20 and 24 weeks after treatment.

At sampling, each lemon was rinsed with 200 ml of acetone to remove the surface residues, then cut into eight slices and peeled and processed through a juice extractor. The peel was diced, and homogenized in liquid nitrogen and dry ice. The TRR level was determined for each type of sample.

All the samples were extracted twice with methylene chloride, and the remaining solids in the pulp and peel samples were extracted twice with a mixture of acetonitrile and 0.1 N hydrochloric acid. The distribution of ^{14}C in each extracted fraction was monitored. The acetone rinses and all organosoluble and aqueous fractions obtained at each sampling were analysed by both TLC and reversed-phase HPLC.

Most of the TRR was found in the peel and rinse at all samplings, with very little in the pulp and juice. Radioactivity in the peel increased from 57% of the TRR at 2 hours to 97% at 2 weeks, and remained at about the same level for the 24-week storage period. Radioactivity in the pulp increased from 0.17% at 2 hours to 3.8% at 24 weeks, and in juice from 0.09% at 2 hours to 2.5% at 10 weeks, maintaining a level of about 2% for the remaining weeks. Surface residues removed by acetone decreased from 43.02% to 0.85% after 24 weeks. Solvent extraction of the peel showed 76%, 23% and 1.15% of the peel radioactivity in methylene chloride, aqueous acetronitrile, and post-extraction solid (PES) fractions respectively 2 hours after treatment, and 19.9, 73.5 and 6.6% respectively 24 weeks after treatment. The corresponding proportions in the pulp were about 31-38%, 54-62% and 6-8%, and in juice about 32-44%, 29-33% and 26-35%.Table 6 shows the distribution of ^{14}C in the rinse, peel, pulp and juice at each sampling.

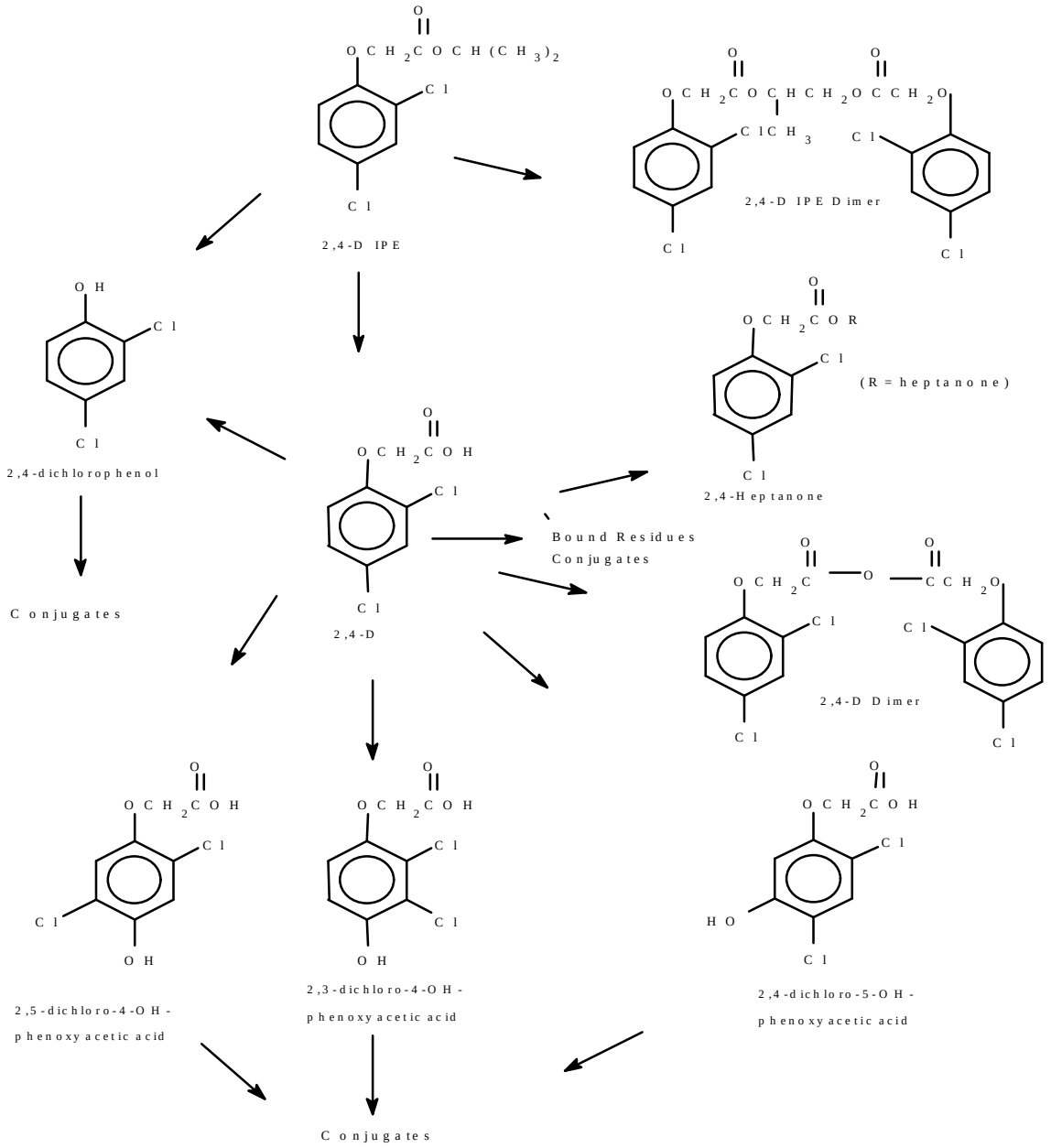
Table 6. Distribution of radioactivity in lemons at various sampling intervals (Wu, 1994).

Sampling	TRR, mg/kg as 2,4-D	Pan wash & rinse		Juice		Pulp		Peel	
		% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
2 h	2.8	43	1.2	0.09	0.003	0.17	0.005	56.7	1.6
2 days	2.3	4.9	0.11	0.10	0.002	0.20	0.005	94.8	2.2
7 days	2.3	3.2	0.07	0.31	0.007	0.55	0.01	96.0	2.2
14 days	2.3	2.1	0.05	0.51	0.01	0.66	0.02	96.8	2.3

Sampling	TRR, mg/kg as 2,4-D	Pan wash & rinse % of TRR	mg/kg	Juice % of TRR	mg/kg	Pulp % of TRR	mg/kg	Peel % of TRR	mg/kg
28 days	2.1	1.6	0.03	0.93	0.02	1.3	0.03	96.1	2.0
6 weeks	2.2	1.2	0.03	1.2	0.03	1.7	0.04	95.9	2.2
8 weeks	2.3	1.1	0.03	1.8	0.04	2.3	0.05	94.8	2.2
10 weeks	2.4	1.1	0.03	2.5	0.06	3.1	0.07	93.3	2.3
12 weeks	2.4	1.0	0.025	1.9	0.05	2.7	0.07	94.4	2.3
16 weeks	2.3	0.80	0.02	1.5	0.035	2.3	0.06	95.4	2.2
20 weeks	2.3	0.93	0.02	2.0	0.05	3.5	0.08	93.5	2.1
24 weeks	2.4	0.85	0.02	1.9	0.05	3.8	0.09	93.4	2.3
Average	2.34								

Metabolites were determined by HPLC and TLC with radiometric detection. Most of the low-level residues (0.64% of the TRR, 0.015 mg/kg) in the 20-week rinse were detected at HPLC retention times of about 36-50 minutes. Four metabolites were tentatively identified by GC-MS: a heptanone ester of 2,4-D, a 2,4-D dimer postulated as 2,4-D anhydride, a dimer of 2,4-D IPE, and a substituted 2,4-dichlorophenol. 2,4-D (0.12% of the TRR, 0.003 mg/kg) and 2,4-D IPE (0.05% of the TRR, 0.001mg/kg) were also found in the rinse. The lemon peel at 20 weeks contained 93.5% of the TRR (2.1 mg/kg). Most of these residues consisted of free and conjugated forms of 2,4-D (64% of the TRR, 1.45 mg/kg). Other metabolites found in minor quantities were free and bound 2,4-D IPE (0.73% of the TRR, 0.017 mg/kg), 4-hydroxy-2,3-D or 5-hydroxy-2,4-D (0.58%, 0.013 mg/kg), 4-hydroxy-2,5-D (0.44%, 0.01 mg/kg) and 2,4-dichlorophenol (0.72%, 0.016 mg/kg). Small amounts of ester-like metabolites similar to those found in the rinses were detected (0.92% of the TRR, 0.02 mg/kg). The main metabolites found in the pulp and juice were also free and conjugated 2,4-D (2.9% of the TRR, 0.07 mg/kg, in the pulp, and 0.99% of the TRR, 0.02 mg/kg, in the juice).

Figure 2. Proposed metabolic pathways of 2,4-D IPE in stored lemons (Wu, 1994).



0 Proposed Metabolic Pathways of 2,4-D IPE in Stored Lemons

The post-extraction solid fraction PES-1 (5.3% of the TRR, 0.12 mg/kg) from the 20-week peel was hydrolysed with cellulase, then 1 *N* HCl and finally 6 *N* HCl. Each hydrolysate was partitioned with ethyl acetate (EtOAc). The organic and aqueous fractions, as well as the final PES, were monitored for ^{14}C. Cellulase released about 27% of the PES radioactivity; 17.6% was soluble in EtOAc (EtOAc-1), and 9.4% remained in the aqueous fraction (Aq-1). Hydrolysis of the resulting PES-2 with *N* HCl released 13.2% of the radioactivity; 10.2% was soluble in EtOAc (EtOAc-2), and 3% remained in the aqueous fraction (Aq-2). Hydrolysis of the remaining PES-3 with 6 *N* HCl released 43% of the radioactivity, 39.5% soluble in EtOAc (EtOAc-3) and 3.7% remaining in the aqueous fraction (Aq-3), while 57% remained in final PES-4. Owing to the low residue levels only the EtOAc-1 fraction was analysed by HPLC and two-dimensional TLC. Four metabolites were detected in this fraction; the main metabolite (about 89% of EtOAc-1, <0.1% of the TRR) had a similar TLC R_f value to 2,4-D, but the HPLC retention times did not match.

Potatoes. [^{14}C]2,4-D ethylhexyl ester was applied as an over-the-top spray to the immature foliage of potato plants grown in a greenhouse (Puglis and Smith, 1992). The diluted emulsified concentrate was applied twice at 0.067 kg acid equivalent (ae)/ha, the first application to three to four week-old plants and the second approximately one week later, according to the registered use of the herbicide on potatoes. Phytotoxicity was observed on the foliage, and the tuber yield was reduced by 56% compared to the control plant. The specific activity of the formulated [^{14}C]2,4-D ethylhexyl ester was 4.08 mCi/mmol (41,000 dpm/µg ae), and the radiochemical purity was 99%. The tubers were harvested at maturity 82 days after the last application. The TRR in the tubers was determined by combustion of tissue aliquots to $^{14}CO_2$ and subsequent radio-analysis. Aliquots of the ground potato tubers were extracted by two methods, one to determine free residues and the other total free and acid-hydrolysable residues. The free residues were isolated by extraction of a 5 *N* hydrochloric acid slurry of the ground potato tuber with diethyl/ether petroleum ether (3:1). The total free and acid-hydrolysable residues were isolated after heating a 5 *N* hydrochloric acid extract of the ground tuber with 5 *N* sulfuric acid and extracting the hydrolysate with the ether mixture (Table 7).

Table 7. Distribution of ^{14}C residues in potato tubers (Puglis and Smith, 1992).

Total ^{14}C, mg/kg as 2,4-D	Free ^{14}C, %		Sample of free and acid-hydrolysable ^{14}C, %	
	aqueous	ether	aqueous	ether
0.0054	46	17	65	30

The total ^{14}C residue in the potato tubers was very low. In an attempt to characterize the organosoluble residue, a 50 g sample was subjected to acid hydrolysis followed by extraction with diethyl ether. The ^{14}C activity in the partially purified extract was observed at an R_f value that did not match the R_f of either the 2-ethylhexyl ester or the free acid. The unknown ^{14}C-labelled compound from the TLC zone was fortified with unlabelled 2,4-D acid and reanalysed by TLC. The added 2,4-D was found at the same R_f value as the unknown compound, and the identity was confirmed by mass spectrometry and high-performance liquid chromatography. Thus, if 2,4-D were present in the treated tubers, it would chromatograph in the same region as the unknown ^{14}C activity. Further characterization of the unknown ^{14}C component(s) was not possible owing to the very low concentration (<0.001 mg/kg 2,4-D ae) and the significant interference of other sample components with the chromatography. An exaggerated application rate was not used to achieve higher residue levels in the tubers because an unacceptable phytotoxic response to rates above 0.067 kg ae/ha.

A second study was conducted with [^{14}C]2,4-D EHE formulated as an emulsifiable concentrate solution (Premkumar and Vengurlekar, 1994). Two foliar applications were made to the plants, the first when the tubers were about the size of a pea 58 days after planting, the second 14 days later. The average radiochemical purity of the formulated solution was 95.4%, and the specific activity 1.55 x 10^5 dpm/mg 2,4-D acid equivalents. The formulated [^{14}C]2,4-D EHE was applied at an average exaggerated rate of 0.35 kg acid equivalents/ha (0.33 and 0.37 kg ae/ha for the first and second applications respectively), 4.4 times the maximum label rate of 0.078 kg ae/ha.

Foliage, vine and tuber samples were analysed for ^{14}C. The vines sampled 1, 7 and 20 days after the second application had residues of 15, 10 and 6.3 mg/kg as 2,4-D respectively, and the tubers collected at the same times contained total ^{14}C residues of 0.32, 0.65 and 0.58 mg/kg respectively. Because the watering of the plants after application was directed to the soil to avoid wash-off from the foliage, these results suggest that the [^{14}C]2,4-D EHE and/or its metabolites were translocated from the plant tops to the tubers.

The ^{14}C residues in the mature tubers were extracted with acidified acetonitrile, which extracted 105% of the TRR (0.61 mg/kg) and 5.4% (0.031 mg/kg) remained unextracted. HPLC of

the extracts showed that 8.3% of the TRR (0.048 mg/kg) was the parent 2,4-D EHE. The main identifiable metabolite was 2,4-D (46% of the TRR, 0.27 mg/kg) and the remaining radioactivity was distributed among four or more polar components. When the extract was concentrated and re-analysed by HPLC the profile changed considerably in the polar region and the parent was found to be unstable during the concentration process, being reduced to 1.6% of the TRR, 0.009 mg/kg. Also the 2,4-D concentration decreased to 36.4% of the TRR (0.21 mg/kg).

The extraction, concentration and analysis procedure was validated by fortifying control samples with 2,4-D EHE at 0.62 mg/kg. Upon chromatographic analysis, 95% of the parent compound remained unchanged. The remainder was hydrolysed to 2,4-D (3.4%) and an unknown component (1.9%).

The treated tubers were then extracted with acetonitrile. This extracted 96% of the TRR (0.55 mg/kg) and 9% (0.052 mg/kg) remained in the post-extraction solids (PES). HPLC analysis of the acetonitrile extract demonstrated that 0.5% of the TRR (0.003 mg/kg) was present as 2,4-D EHE, and 40% (0.23 mg/kg) as free 2,4-D. The remaining radioactivity was distributed among several polar components. During concentration and analysis some parent was again lost and changes were observed in the polar components but the 2,4-D remained unchanged (43% of the TRR, 0.25 mg/kg) indicating that the parent ester and some polar components (perhaps conjugates) were not stable during concentration.

The extracted ^{14}C residues were subjected to acid hydrolysis. HPLC of the hydrolysate showed three major compounds which were tentatively identified as 4-hydroxy-2,5-D (15% of the TRR, 0.084 mg/kg), 4-chlorophenoxyacetic acid (4-CPAA; 24% of the TRR, 0.14 mg/kg) and 2,4-D (39.5% of the TRR, 0.23 mg/kg). The amount of 2,4-D in the extract remained unchanged after hydrolysis, suggesting that the polar components in the extract could be conjugates of 4-hydroxy-2,5-D and 4-CPAA. All three major products were isolated and methylated: GC-MS of the methyl esters confirmed the identification of 4-hydroxy-2,5-D, 4-CPAA and 2,4-D.

Acid hydrolysis released some bound ^{14}C residues from the PES (9.0% of the TRR, 0.052 mg/kg) and the pellet remaining after the concentration of the acetonitrile extract (4.9% of the TRR, 0.028 mg/kg). The acetonitrile phase contained 8.7% of the TRR (0.05 mg/kg) and 6.4% of the TRR (0.037 mg/kg) remained unextracted. HPLC radio-analysis of the released ^{14}C residues demonstrated the presence of 2,4-D (2.3% of the TRR, 0.013 mg/kg), 4-CPAA (1.6% of the TRR, 0.009 mg/kg) and 4-hydroxy-2,5-D (0.9% of the TRR, 0.005 mg/kg). The components were characterized on the basis of their retention times. A total of 1.4% of the TRR (0.008 mg/kg) was eluted in the void volume and remained unidentified.

Figure 3. Metabolic pathways of 2,4-D EHE in potato tubers (Premkumar and Vengurlekar, 1994).
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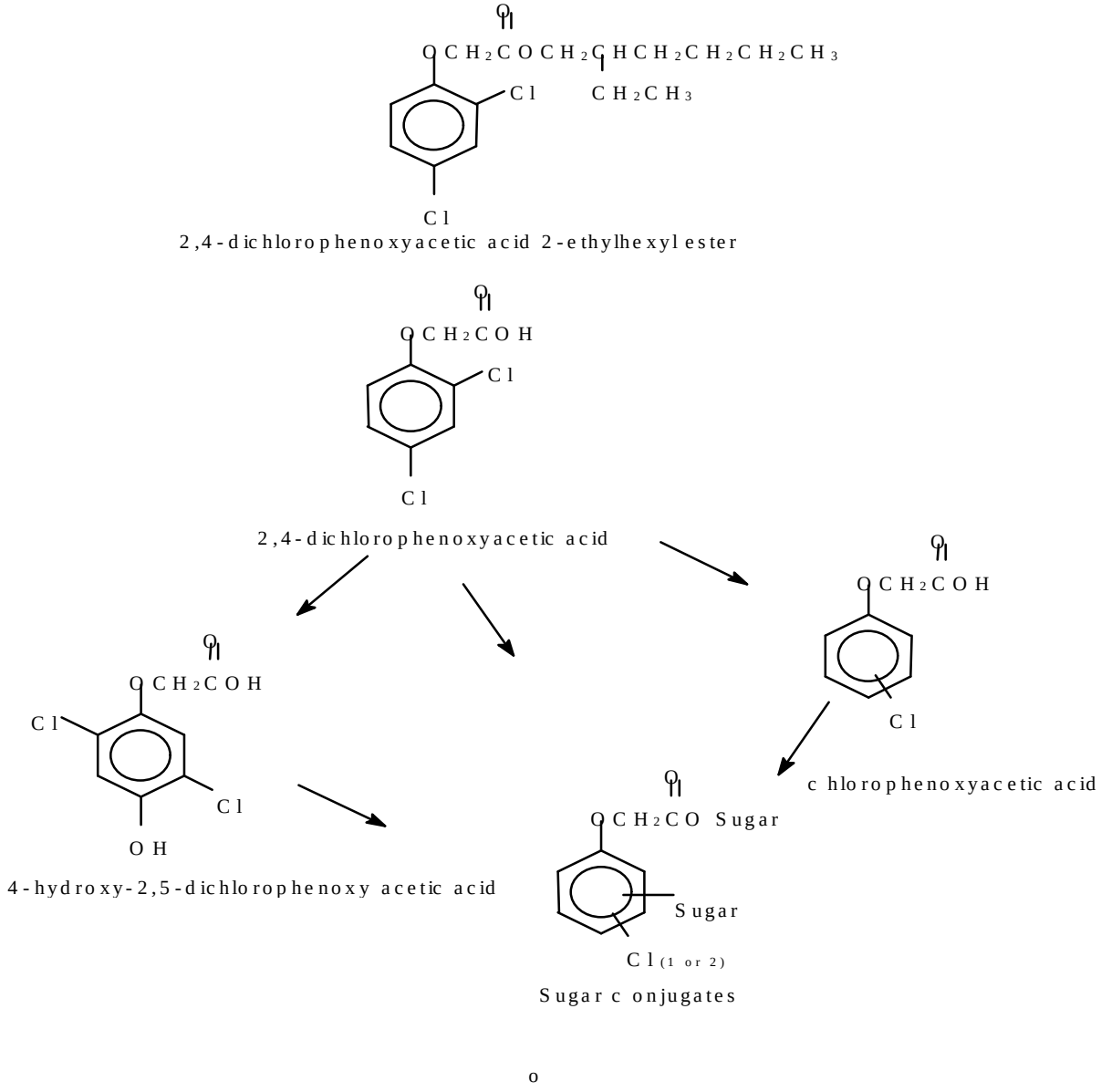

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The distribution of the ^{14}C components is shown in Tables 8 and 9.

Table 8. Distribution of ^{14}C residues (0.58 mg/kg) in extracts of mature potato tubers treated with 2,4-D EHE (Premkumar and Vengurlekar, 1994).

Residue component	Acidified acetonitrile extract		Acetonitrile extract	
	% TRR	mg/kg[1]	% TRR	mg/kg[1]
Polar unknown[2]	27.7	0.16	9.5	0.055
4-hydroxy-2,5-D	18.4	0.11	6.4	0.04
4-CPAA	1.0	0.006	4.3	0.025
2,4-D	46.3	0.27	40.4	0.23
2,4-D 2-EHE	8.3	0.05	0.5	0.003

Residue component	Acidified acetonitrile extract		Acetonitrile extract	
	% TRR	mg/kg[1]	% TRR	mg/kg[1]
Unidentifiable	3.3	0.019	28.6	0.17
Unaccounted for	-	-	6.0	0.03
Unextractable	5.4	0.031	9.0	0.05
Total	110.3	0.64	104.7	0.6

[1] 2,4-D acid equivalents
[2] Eluting in the void volume

Table 9. Distribution of ^{14}C residues in acid hydrolysates of unextracted ^{14}C residues (Premkumar and Vengurlekar, 1994).

Residue component	CH_3CN extract of acid hydrolysis		Post extraction solid (PES) from acid hydrolysate		Total in tuber	
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
4-Hydroxy-2,5-D	14.6	0.08	0.9	0.005	15.5	0.09
4-CPAA	24.4	0.14	1.6	0.009	26.0	0.15
2,4-D	39.5	0.23	2.3	0.01	41.8	0.24
Unidentified	8.5	0.05	3.9	0.02	12.4	0.07

Wheat. In a wheat metabolism study [^{14}C]2,4-D EHE formulated as an emulsifiable concentrate solution was applied in a single over-the-top spray to spring wheat, at about the tiller stage, at 1.68 kg ae/ha which is in excess of the maximum label rate (Puvanesarajah, 1992). The radio purity of the formulated solution was 98% and the specific activity was 20,070 dpm/µg ae (2.0 mCi/mmol). The plants were grown under partially enclosed greenhouse conditions.

The TRR levels, as 2,4-D equivalents, in the forage, straw and grain samples were determined by combustion radio-analysis and liquid scintillation counting (LSC). The ^{14}C in the forage, straw and grain was extracted with organic and aqueous/organic solvents and 0.5 M KOH in aqueous methanol. The remaining residues were treated with enzymes. Diethyl ether and aqueous ethanol extraction recovered a large proportion of the residues in the forage and straw. Most of the residues recovered from the grain were in the aqueous and aqueous/methanolic KOH extracts. The distribution of the ^{14}C is shown in Table 10.

Table 10. Wheat. Distribution of ^{14}C in extraction solvents (Puvanesarajah, 1992).

Sample	Total ^{14}C, mg/kg[1]	% in ether	% in EtOH	% in AcN/H_2O	% in H_2O	% in KOH	% in enzyme digest	% in Aq. HCL	% un-extracted
10-day forage	34	20	66	N/A	N/A	12	0.04		0.4
49-day straw	56	14	63	N/A	N/A	19	1.7		0.4
49-day grain	0.30	N/A	N/A	3.7	7.7	28	10	22	8.9

[1] As 2,4-D ae

The radioactive residues in the extracts were separated by HPLC. Owing to the many metabolites and conjugates, quantification of the components by direct HPLC analysis of the extracts was not possible. Base and acid hydrolyses of the ether and ethanol extracts converted most of the metabolites to exocons which were separated by HPLC and their identities confirmed by MS and/or TLC (Table 11).

Table 11. Distribution of the components of extractable residues in wheat (Puvanesarajah, 1992).

Sample	Total ^{14}C, mg/kg [1]	% of Total ^{14}C extracts[4]					
		2,4-D EHE	2,4-D	2,4-dichloro phenol	Hydroxydichloro-phenoxyacetic acids[5]	In natural constituents	Not identified[6]
10-day forage	34	3.1 [2]	74 [2]	0.5 [3]	8 [3,]		9.8
49-day straw	56	2.0 [2]	70 [2]	0.9 [3]	8.5 [3]		12
49-day grain	0.3	-	6	-	-	45 [7]	41

[1] As 2,4-D acid ae
[2] Identity confirmed by MS, TLC and HPLC
[3] Identity confirmed by MS and HPLC
[4] Total of free and conjugated
[5] 4-Hydroxy-2,5-dichlorophenoxyacetic acid and two other structural isomers H_2 & H_3
[6] In general, this radioactivity is associated with many different fractions over a wide range of retention times and/or with radioactivity which was unaccounted for after HPLC analysis. Thus, no single unidentified component is present at the level shown.
[7] Incorporated into protein, starch and cellulose fractions

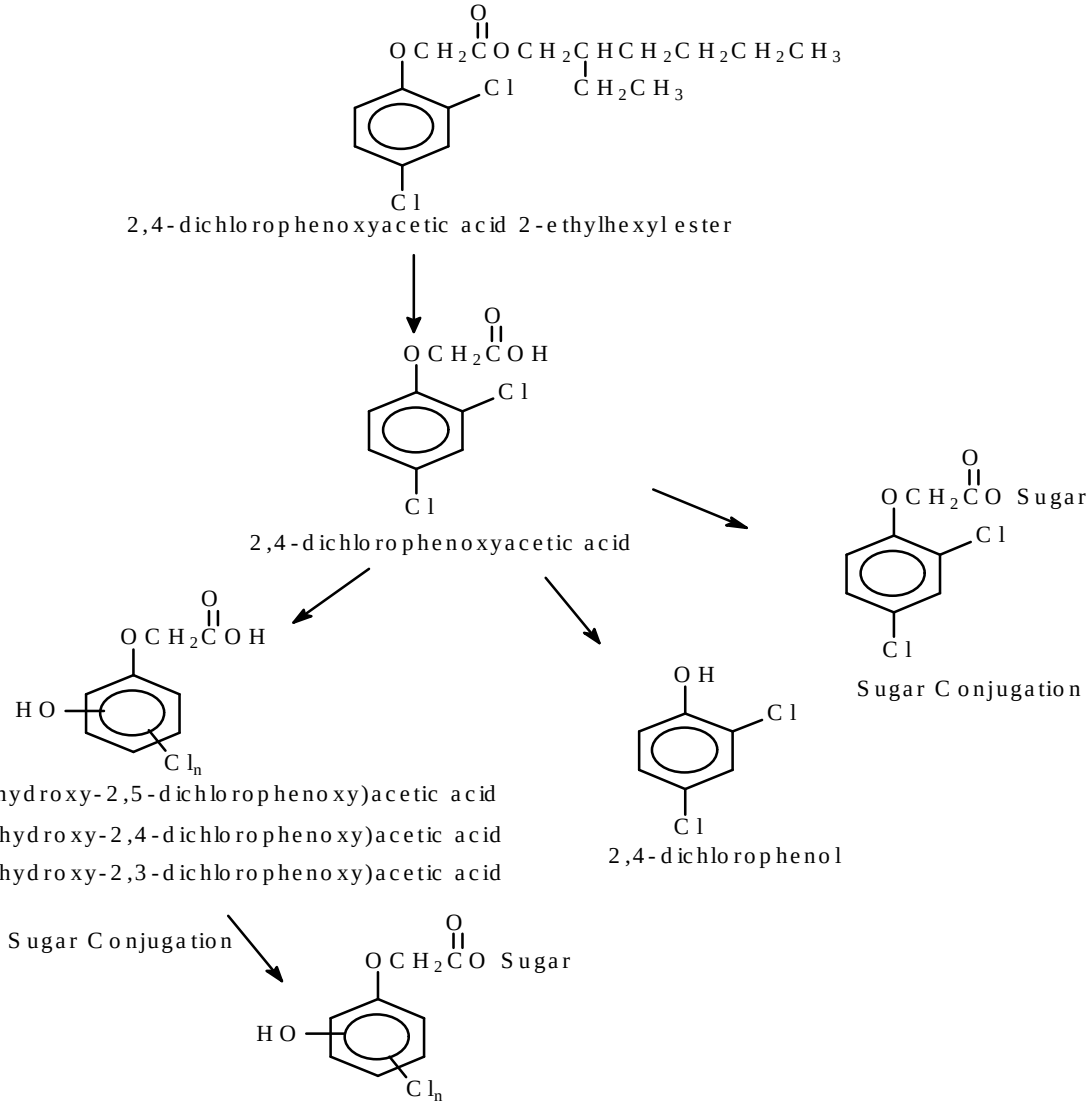
In forage and straw unconjugated 2,4-D was found at 9% and 6% respectively, and base-labile 2,4-D conjugates accounted for 64% of the total residues. The hydrolysis of these 2,4-D conjugates by a mild base suggests esterification with indigenous substances, presumably sugars. Approximately 33% of the total ring-hydroxylated 2,4-D derivatives identified in the forage were free residues extractable in ether, and the others were found as polar conjugates in the ethanol extract. These polar conjugates were stable to bases but were readily cleaved under acidic conditions, which suggests they were phenolic glycosides. A large portion (about 77%) of the ring-hydroxylated metabolites in the straw were polar conjugates. Side-chain degradation of 2,4-D to give 2,4-dichlorophenol was a minor pathway (see Table 11).

2,4-D residues identified in the grain extracts represented $\leq$6% of the total grain residues. 45% from the incorporation of ^{14}C into proteins, starch and cellulose. Untreated control samples had residues of 0.17 mg/kg. This ^{14}C contamination was attributed to the proximity of the control and treated wheat to another plot in which $^{14}CO_2$ was generated from the microbial breakdown of 2,4-D EHE applied to soil. Both the treated and control samples were analysed by the same schemes for the extraction of residues and isolation of natural products to distinguish between the residues resulting from treatment of wheat with [^{14}C]2,4-D EHE and that resulting from the uptake of $^{14}CO_2$. These parallel analyses demonstrated that the presence of ^{14}C in the control grain samples did not affect the conclusions on the nature of the residue in wheat grain after treatment of young wheat plants with [^{14}C]2,4-D EHE.

In later work on samples from the above study (Pither, 1992) the two isomeric hydroxydichlorophenoxyacetic acids reported as H_2 and H_3 in wheat forage and straw (at 1.4 to 2.5% of the total ^{14}C residue) were identified from their chromatographic mobilities and mass spectral data as 4-hydroxy-2,3-dichlorophenoxyacetic acid and 5-hydroxy-2,4-dichlorophenoxyacetic acid. These compounds had HPLC elution ranges of 15.0-17.5 min and 18.5-21.5 min respectively. The presence of the ^{14}C components with HPLC elution ranges similar to those of 2,4-D EHE and 2,4-D in the basic extract of control wheat grain was re-examined, and the presence of 2,4-D in the extracts of control and treated wheat grain was confirmed by the re-analysis. 2,4-D EHE could not be detected

in the basic extract from control wheat grain when precautions were taken to eliminate contamination by carry-over during HPLC analysis.

Figure 4. Metabolic pathways of 2,4-D EHE in spring wheat (Puvanesarajah, 1992; Puvanesarajah and Ilkka, 1992).



0 Metabolic Pathway for 2,4-D 2-EHE in Spring Wheat

Environmental fate in soil

Aerobic degradation

Concha. and Shepler (1994a) studied the aerobic degradation of [^{14}C]2,4-D in unsterilized Catlin silty clay soil applied at a rate of 5.1 mg/kg for 16 days. The samples were incubated at about 25°C throughout the study. Recoveries of radiocarbon averaged 95.6 ± 6.3%.

The 2,4-D was degraded rapidly and represented 0.5% of the applied dose after 16 days of exposure. The calculated half-life was 1.7 days based on pseudo-first order kinetics.

The main degradation product was CO_2, which accounted for 51.2% of the applied dose at the end of the study period. Two major products were 2,4-DCP (2,4-dichlorophenol) and 2,4-DCA (2,4-dichloroanisole) which rose and fell over the 16-day period. 2,4-DCP reached its maximum of 3.5% of the applied dose at day 2, then decreased to 0.4% at the end of the study period, while 2,4-DCA represented 2.5% of the applied dose at day 9 and decreased to 1.5%; and the unextractable residue was 36% of the TRR. Separation of the fulvic and humic acid fractions of an extracted sample at day 5 afforded 16% of the applied dose in the former and 11% in the latter. Further analysis of the fulvic acid fraction by HPLC recovered 6.1% of the applied dose as 2,4-D.

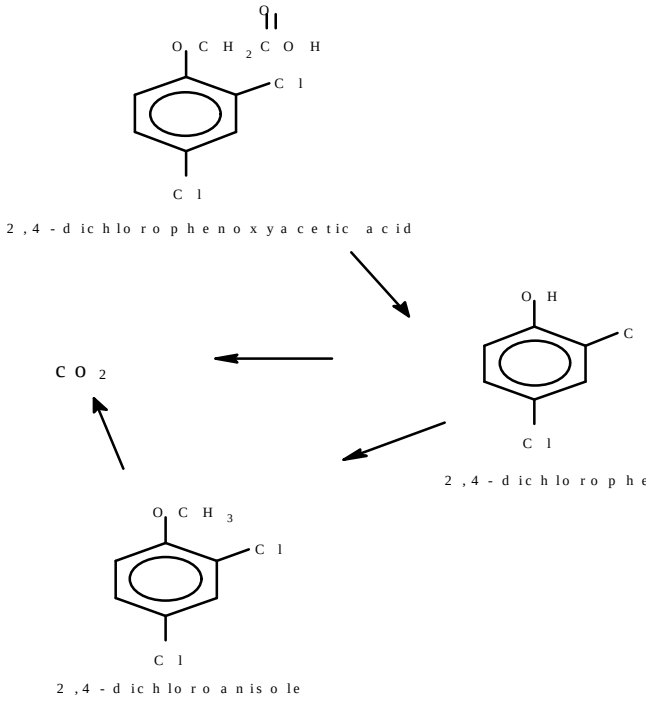
The results indicate that 2,4-D and its degradation products should dissipate rapidly from the soil environment mainly by mineralization and incorporation into the soil organic matter.

Reynolds (1994) determined the fate of the 2-ethylhexyl moiety of 2,4-D EHE by incubating ^{14}C-labelled 2-ethylhexanol in an aerobic soil system at 10 mg/kg under laboratory conditions at 25 ± 1°C in the dark. Sampling was at 0, 2, 4, 8, 12, 18, 24, 48, 168 and 336 hours after treatment.

Degradation was rapid. Initially most of the applied radioactivity was extracted into the organic solvent (methanol/methylene chloride) but the percentage of ^{14}C extracted decreased rapidly from 91% to 13% during the first 48 hours. By days 7 and 14, the organic extract accounted for 6.9% and 6.4% of the total applied radioactivity. The percentage of ^{14}C in the post-extraction solids (PES) remained fairly constant for the first 8 hours then began to increase dramatically, reaching its highest level (48% of the applied ^{14}C) at 48 hours. By day 14 the ^{14}C in the PES accounted for 22% of the total applied radioactivity.

During the first 24 hours the evolved acidic volatiles, including $^{14}CO_2$, increased gradually. Between 24 and 48 hours, however, the level detected in the KOH solution had quadrupled to 47%. Volatile ^{14}C accounted for a maximum of 70% of the applied ^{14}C on day 14.

Figure 5. Proposed degradation pathways of 2,4-D in soil (Concha and Shepler, 1994a).



Recoveries of ^{14}C from days 0 to 14 ranged from 93% to 108%. The average overall recovery of the applied radioactivity was 99%.

Analyses of the organosoluble fraction by reversed-phase HPLC showed one main product, which reached a maximum of 84% at 18 hours, in addition to 2-ethylhexanol. This was identified by GC-MS as 2-ethylhexanoic acid. Low levels of 5 other compounds were observed at later sampling times, but none of these exceeded 2.6% of the total applied radioactivity.

The PES fraction from day 14 was subjected to various procedures which included shaking for 4 hours with acetonitrile/water/acetic acid and refluxing for 1 hour with 0.25 *N* HCl, followed by shaking with 0.5 *N* NaOH for 24 hours to release the bound residues. The radioactivity extracted by $CH_3CN/H_2O/HOAc$ accounted for 1.6% of the total; 4% could be extracted from the solids following reflux with 0.25 *N* HCL for about 1 hour. The radioactivity that was extracted into the acid hydrolysate remained mostly in the aqueous fraction after partitioning with EtOAc. Less than 0.38% of the total applied radioactivity was detected in the EtOAc fraction. Most of the radioactivity in the solids appeared to be incorporated into natural soil constituents (fulvic acid, humic acid and humins). The ^{14}C in the fulvic and humic acids and humins was 4.2%, 4.6% and 7.5% of the total applied radioactivity respectively.

The half-life of 2-ethylhexanol in soil under the aerobic test conditions at 25°C was calculated to be 5.3 hours.

Reynolds (1995e) investigated the fate of the diethanolamine moiety from the diethanolamine salt by incubating diethanolamine with soil (10 mg/kg) under identical conditions to the above. [^{14}C]diethanolamine labelled on the 1-carbons was degraded rapidly with a calculated half-life in sandy loam soil of 1.35 days.

Analyses were by HPLC and TLC comparisons and LSC.

The parent compound steadily decreased throughout the study with the Hanford sandy loam soil. Two days after dosing, less than 38% of the total applied radioactivity was attributable to the parent compound. Its level had decreased to 0.9% of the applied ^{14}C by day 14 and to less than 0.6% at 90 days.

A total of ten products (M1-M10) were observed during the 90-day study. M2 accounted for 9.91% of the total applied radioactivity at day 0 but was not detected at day 1. Low levels of M2 appeared at day 2, reached a maximum of 1.4%, but then decreased to 0.1%.

The main product was M3 which, as determined by reversed-phase HPLC, reached 7.9% at day 2 and then steadily decreased to less than 1% at the end of the study. TLC analysis of the same fraction indicated that M3 consisted of three or four compounds, none of which exceeded 3% of the applied radioactivity.

Low levels of glycine (M4) and ethanolamine (M5) were also detected. Glycine reached a maximum of 5% at day 3, but at 90 days was less than 0.4%. Ethanolamine did not exceed 2.6% of the total radioactivity.

None of the products M6, M7, M8, M9 or M10 exceeded 0.8% of the dosed ^{14}C.

Up to 60% of the dosed ^{14}C in the sandy loam soil was converted to CO_2. The levels of bound residues reached a maximum of 33% at day 7. At day 90, the level of radioactivity detected in the post extraction solids (PES) accounted for 24% of the total applied ^{14}C.

The PES fraction from day 7 was subjected to acid hydrolysis for 4 hours followed by a 24-hour shaking with 0.5 *N* NaOH. Acid hydrolysis released 20% of the ^{14}C. Less than 1% of the radioactivity extracted by 0.25 *N* hydrochloric acid could be partitioned into ethyl acetate. TLC analysis of the aqueous fraction showed five or six compounds. The remaining radioactivity in the solids was incorporated into fulvic acid (4.4%), humic acid (3.7%) and humins (5%), indicating that diethanolamine is incorporated into the natural soil constituents and then mineralized to CO_2.

Adsorption/desorption

Cohen (1991b) determined the adsorption/desorption of unaged [^{14}C]2,4-D in unsterilized Louisiana rice paddy sediment by the batch equilibrium technique.

Two preliminary studies were conducted to determine the adsorption and desorption equilibrium times (24 and 8 hours respectively). It was also demonstrated that 2,4-D acid does not bind to glass surfaces.

A definitive study was conducted at five different concentrations (0.1, 0.51, 1, 2.47 and 5.02 mg/kg) at 22 ± 1°C. The K_d value for 2,4-D acid was 1.22 (K_{oc} = 58.1) indicating adsorption to sediment from water during the adsorption phase of the study. However, in the desorption phase, the K_d value of 1.64 (K_{oc} =78.1) indicated that 2,4-D is moderately to highly mobile in rice paddy sediment. The average ^{14}C recovery for the five concentrations tested was 98.9%.

Fathulla (1996b) studied the adsorption and desorption characteristics of [^{14}C]2,4-D on four representative agricultural soils. Samples were prepared at a soil:solution ratio of 1g:1ml, with nominal concentrations of [^{14}C]2,4-D in aqueous 0.01 *M* calcium chloride of 10, 5, 2.5 and 1 mg/ml. The samples were equilibrated in a shaking water bath for 24 hours at 25 ± 1°C, followed by vortexing and centrifugation. ^{14}C was measured in the resulting supernatant by LSC to determine the adsorption of the test material to the soil. The supernatant was then removed from each sample and replaced with an equal volume of untreated 0.1 *M* $CaCl_2$. The samples were equilibrated for 24 hours

at 25 ± 1°C, followed by vortexing and centrifugation. ^{14}C in the supernatant was determined by LSC and the radioactivity remaining in the soil after desorption was determined by oxidation followed by LSC.

Linear regression analysis of the adsorption and desorption data for all the soils demonstrated that adsorption and desorption of [^{14}C]2,4-D followed the Freundlich equation. The adsorption and desorption equilibrium constants ($K_{d\text{-}a}$ and $K_{d\text{-}d}$ respectively), and coefficients $K_{oc\text{-}a}$ and $K_{oc\text{-}d}$ are shown in Table 12.

Table 12. Adsorption and desorption data of 2,4-D in soils (Fathulla, 1996b).

	Adsorption		Desorption	
Soil	$K_{d\text{-}a}$	$K_{oc\text{-}a}$	$K_{d\text{-}d}$	$K_{oc\text{-}d}$
Plainfield sand	0.36	76	1.16	247
California sandy loam	0.17	70	0.81	338
Mississippi loam	0.28	117	1.48	617
Arizona silty clay loam	0.52	59	1.90	216

The test material was largely stable in the test system in all the soils. After 24 hours, the total amounts of unchanged [^{14}C]2,4-D in the adsorption solution and soil extract were 95% of the original for Arizona silty clay loam, 109% for California sandy loam, 97% for Mississippi loam, and 98% for Plainfield sand. [^{14}C]2,4-D was stable over a 24-hour period and in aqueous 0.01 *M* $CaCl_2$, 100% of radioactivity in the desorption solution was owing to unchanged [^{14}C]2,4-D after 48 hours,.

The potential for [^{14}C]2,4-D to leach though soil, based solely on estimations of relative mobility using $K_{oc\text{-}a}$ values, is high for all the soils tested.

Fathulla (1996c) determined the adsorption and desorption characteristics of the soil degradation product 2,4-dichloroanisole (2,4-DCA) on the same four soils. Samples were prepared at a soil:solution ratio of 1g:5ml with nominal concentrations of [^{14}C]2,4-DCA in aqueous 0.01 *M* calcium chloride of 5, 2.5, 1 and 0.5 mg/ml. Other experimental details were as described above (Fathulla, 1996b).

Linear regression analysis of the data demonstrated that the adsorption and desorption of [^{14}C]2,4-DCA followed the Freundlich equation. The adsorption and desorption equilibrium constants and coefficients are shown in Table 13.

Table 13. Adsorption and desorption of 2,4-DCA in soils (Fathulla, 1996c).

	Adsorption		Desorption	
Soil	$K_{d\text{-}a}$	$K_{oc\text{-}a}$	$K_{d\text{-}d}$	$K_{oc\text{-}d}$
Plainfield sand	2.05	436	3.4	721
California sandy loam	1.6	667	2.4	996
Mississippi loam	3.46	1,442	4.5	1,867
Arizona silty clay loam	5.42	616	8.6	975

The test material was again stable in all the test systems. After 24 hours, the total amounts of unchanged [^{14}C]2,4-DCA in the adsorption solution and soil extract were 94% of the original for

Arizona silty clay loam, 105% for California sandy loam, 105% for Mississippi loam and 103% for Plainfield sand. After 48 hours 100% of the radioactivity in the desorption solution was due to unchanged [^{14}C]2,4-DCA.

The potential for 2,4-DCA to leach though the four soils, on the basis of the K_{oc-a} values is medium to low.

Fathulla (1996d) also studied the adsorption and desorption characteristics of 2,4-dichlorophenol in the four agricultural soils. Samples were prepared at a soil:solution ratio of 1g:2ml, with nominal concentrations of [^{14}C]2,4-DCP in aqueous 0.01 *M* calcium chloride of 10, 5, 2.5 and 1 mg/ml. Other experimental details were as described above (Fathulla, 1996b).

The adsorption and desorption of [^{14}C]2,4-DCP again followed the Freundlich equation. The adsorption and desorption equilibrium constants and the adsorption and desorption coefficients are shown in Table 14.

Table 14. Adsorption and desorption of 2,4-DCP in soils (Fathulla, 1996d).

Soil	Adsorption		Desorption	
	K_{d-a}	K_{oc-a}	K_{d-d}	K_{oc-d}
Plainfield sand	1.7	368	3.8	813
California sandy loam	1.97	821	6.3	2,625
Mississippi loam	2.9	1,204	5.6	2,325
Arizona silty clay loam	3.3	374	7.1	807

The test material was largely stable in all four test systems. After 24 hours, the total amounts of unchanged [^{14}C]2,4-DCP in the adsorption solutions and soil extracts were 92.5% of the original for Arizona silty clay loam, 93% for California sandy loam, 92% for Mississippi loam and 92% for Plainfield sand. [^{14}C]2,4-DCP was shown to be stable for 24 hours in aqueous 0.01 *M* $CaCl_2$, and for 48 hours in the desorption solution.

The potential for [^{14}C]2,4-DCP to leach though the tested soils is low, on the basis of the K_{oc-a} values.

Mobility

Burgener (1993) used lysimeters to represent an agricultural ecosystem in which to study the translocation of [^{14}C]2,4-D and its degradation products in a 120 cm soil and the effects of precipitation and vegetation of leaching. Information on the uptake of the pesticide by plants after soil application, as well as the ^{14}C balance after 725 days, was also obtained.

Two lysimeters were covered with summer wheat. On 15 June 1990, the herbicide was applied as the DMA salt at its recommended field rate, 750 g ae/ha. Husbandry and crop rotation followed common agricultural practice. Winter rye was sown in November 1990, followed by winter rape after harvest of the rye in summer 1991.

Water samples were taken regularly and ^{14}C was determined by LSC to estimate the contribution of dissolved $^{14}CO_2$ from the mineralization of the test compound. The remaining radioactivity was characterized by chromatographic techniques. The radioactivity taken up by the plants as well as that in the soil at the conclusion of the experiment was determined by combustion, while $^{14}CO_2$ and other volatile substances emitted to the atmosphere were calculated by difference.

In lysimeter 1 the ^{14}C recovered in the leachate after 2 years was 0.196% of the total applied radioactivity, corresponding to 0.14 mg parent equivalents. The total mean recovery of radioactivity, all leachate samples included, represented a concentration of 0.14 mg parent equivalents/l. After subtraction of $^{14}CO_2$, which represented 0.099% of the applied radioactivity, the remaining concentration was reduced to 0.07 mg/l. During the whole experimental period, 958 l of water (about 48% of the total precipitation, including irrigation) had been collected.

In lysimeter 2 the radioactivity in the water after two years was 0.25% of the total applied corresponding to 0.17 mg parent equivalents. The total mean recovery of radioactivity, all leachate samples included, represented 0.175 mg parent equivalents/l and 0.058% of the total applied radioactivity was $^{14}CO_2$. After subtracting this, the mean content of ^{14}C as 2,4-D was 0.13 mg/l. During this period, 994 l of water was collected, representing about 50% of the total precipitation, including irrigation.

No volatile substances except for $^{14}CO_2$ were detected in the leachates by TLC or HPLC.

[^{14}C]2,4-D DMA salt, the free acid and its known degradation products 2-chlorophenol, 4-chlorophenol and 2,4-dichlorophenol were not detected in any of the leachates. In both lysimeters, a maximum of three unknown radioactive fractions were detected. The main unknown fraction was present in all the analysed leachate samples. It amounted in total to 0.043% of the applied radioactivity (AR) in lysimeter 1 and to 0.087% in lysimeter 2. The radioactivity evidently originated from very polar residues, and may be assumed in part to be derived from cleaved ring fragments bound to humic and fulvic acids.

At the conclusion of the experiment, soil cores were sectioned into 12 horizontal segments of about 10 cm thickness. In lysimeter 1, the TRR in the soil profile amounted to 21% of the AR, 11% in the top layer. For lysimeter 2 the corresponding proportions were 17% and 8.8%. In both lysimeters, only about 0.1% of the AR was found in the soil below 57 cm.

The extractable radioactivity in the soil again originated from three unknown fractions, the patterns and amounts of which in the soil extracts from the two lysimeters compared well and were nearly identical. Radioactivity was found as deep as 17 cm and amounted in total to 0.26% of the AR in the soil of lysimeter 1 and 0.29% in lysimeter 2. The decomposition products 2-chlorophenol, 4-chlorophenol and 2,4-dichlorophenol were not detectable in any of the six analysed soil layers, which covered a depth of 57 cm.

The water collected during the experiment represented about 50% of the total precipitation. In June 1990, a series of exceptionally heavy rainfalls started one week before the application. Although the total amount in this month was nearly twice the monthly average for the region, the unfavourable conditions did not yield high concentrations of radioactivity.

The number and levels of the compounds found in the leachate and soil samples compared well in the two lysimeters. The results obtained indicated that [^{14}C]2,4-D and its decomposition products are not mobile in the sandy soil used in the experiment and 2,4-D is therefore not considered to have any potential to leach into ground-water. The leached radioactivity originated entirely from very polar unidentified residues.

Uptake by rotational crops

Burnett and Ling (1994) determined the level and nature of the 2,4-D residues taken up by rotational crops from soil treated with [^{14}C]2,4-D, applied by broadcast spray to bare ground on each of three plots at a rate equivalent to 2.2 kg ae/ha. Rotational crops, wheat, icicle white radish and lettuce, were planted 30 days after treatment (DAT) and 139 DAT.

Soil residues decreased significantly during the initial 30 DAT and chromatographic analysis of soil extracts indicated substantial degradation of 2,4-D during this period. Only 8.7% of the soil residues at 30 DAT were soluble in ether compared to an average of 97.5% at 0 DAT. The radioactivity recovered from soil cores at 0 DAT was identified as being from 2,4-D by HPLC, but at 30 DAT only 1.1% of the applied radioactivity was tentatively attributed to 2,4-D.

The TRR as 2,4-D equivalents in the 30 DAT crops except wheat forage ranged from 0.01 mg/kg to 0.06 mg/kg:

Sample	TRR, mg/kg as 2,4-D	
	30 DAT	139 DAT
Radish top	0.043	0.01
Radish root	0.01	0.011
Lettuce	0.019	0.013
Wheat forage	<0.001	0.03
Wheat straw	0.06	0.084
Wheat grain	0.049	0.06

No ether-soluble residues above 0.01 mg/kg were found in any plant samples, whereas in control tissues fortified with 2,4-D more than 65% of the activity was ether-soluble. All samples contained ethanol-soluble residues, but these exceeded 0.01 mg/kg only in radish tops and wheat straw (30 DAT radish tops 0.011 mg/kg, 30 DAT wheat straw 0.017 mg/kg, 139 DAT wheat straw 0.014 mg/kg).

Most of the radioactivity in the plants was not extractable with ether or acidic aqueous ethanol. Hydrolysis of the bound residues solubilized varying amounts of ^{14}C but only traces of ether-soluble residues, none above 0.004 mg/kg. Radioactive components at the retention times of 2,4-D and a hydroxylated product were observed on HPLC analysis of the ether extract of acid-hydrolysed radish tops at [0.001 mg/kg.

In the wheat straw at 30 DAT, crude lignin and cellulose contained 0.01 mg/kg and 0.007 mg/kg respectively. Wheat grain from both planting intervals was shown to contain ^{14}C incorporated into glucose isolated from the starch. The amount of [^{14}C]glucose was consistent with the amount of starch in typical wheat grain (60-70%).

The results showed no ether-soluble residues from free or conjugated 2,4-D or its known wheat metabolites at levels above 0.01 mg/kg after a 30-day planting interval. Extraction, hydrolysis and chromatographic characterization, supported by the detection of ^{14}C in glucose from starch, indicate that the radioactive residues in the rotational crops at both 30 and 139 DAT were owing to natural incorporation.

Terrestrial field dissipation

Fifteen terrestrial field soil dissipation studies were conducted with 2,4-D EHE (Barney, 1995a-d, j; Hatfield, 1995c-j; Silvoy, 1995a,b), and 18 with 2,4-D DMA (Barney, 1995e-i; Burgener, 1993; Hatfield, 1995k-r; Silvoy, 1994a-d). The results were summarized by Wilson *et al.* (1997).

Each test substance was characterized before use. Maximum label rates and treatment sequences were applied to each crop. In some of the trials, test plots were established in bare soil as well as in cropped areas.

In 1993, test sites in Colorado, North Carolina, and Texas (USA) were treated with commercial liquid formulations of 2,4-D DMA or 2,4-D EHE applied as diluted sprays. The Colorado and North Carolina trials included wheat and turf cropping practices with adjacent bare soil treatments, while the Texas plots included pasture cropping.

The wheat and bare soil plots had 1.4 kg ae/ha applied in May and again in July. In Colorado the soil was sandy clay loam with 1.3 to 1.8% organic matter (OM) and pH 7.8 to 8.1 (total precipitation plus irrigation 32 to 36 cm); in North Carolina sand with 0.77 to 1.4% OM, pH 5.4 to 6.9 pH (total precipitation plus irrigation 52 to 86 cm). Turf and adjacent bare soil treatments in North Carolina and pasture treatments in Texas consisted of 2.2 kg ae/ha applied in May and June. In Texas, the soil varied from sandy loam to silt loam with 1.5 to 1.6% OM, pH 6.0 to 6.1 (total precipitation plus irrigation 94cm).

In 1994 the test sites were in California, Nebraska, North Dakota, and Ohio. Sprays of both the amine salt and ester were applied to bare soil following a treatment regime for maize in Nebraska and Ohio, wheat in North Dakota, and pasture, bare soil, and turf in California. A commercial granular formulation of 2,4-D DMA was applied to bare soil and turf in North Dakota, and a 2,4-D EHE granular formulation to bare soil and turf in Ohio.

The maize treatment regime consisted of applications to bare soil of 2.2 kg ae/ha in May, 1.1 kg ae/ha in June, 0.56 kg ae/ha in July, and 1.7 kg ae/ha in September/October. In Nebraska the soil was silt loam with 2.9 to 3.5% OM and pH 5.7 to 6.7. Total precipitation plus irrigation was 79.5 cm. In Ohio the soils included a silty clay loam, clay loam, and silt loam with 2.0 to 5.0% OM and pH 6.5 to 7.1. Total precipitation plus irrigation was 47 to 97 cm. The wheat treatments consisted of 1.4 kg ae/ha applied in June and August. In North Dakota the soils ranged from sandy loam to loam with 2.9 to 6.4% OM and pH 5.9 to 7.7. The total precipitation plus irrigation ranged from 33 to 34 cm. Turf, pasture, and adjacent bare soil treatments consisted of 2.2 kg ae/ha applied in March through July with second applications in April through August. In California, soils varied from a sandy loam to loamy sand with 0.7 to 3.9% OM and pH 6.3 to 7.9. Total precipitation plus irrigation ranged from 67 to 142 cm.

In addition to 2,4-D, the analytical method employed was designed to extract and determine the ester 2,4-D EHE, 2,4-dichlorphenol and 2,4-dichloroanisole. The last two were shown to be relevant in soil degradation studies. The analytes were extracted from soil samples by a combination of three solvent systems and sonication. The combined extracts were diluted with water and concentrated on a C-18 solid-phase cartridge. The analytes were eluted sequentially with two solvent systems that yielded two eluates. The first eluate, containing 2,4-D EHE, 2,4-DCP, and 2,4-DCA, was chromatographed without derivatization. The second contained 2,4-D which was methylated with BF_3/methanol and partitioned into hexane. The first eluate and the hexane solution were combined for analysis by GC-MS. Recoveries of 2,4-D from fortified soil ranged from 91% at 0.01 mg/kg to 76% at 10 mg/kg with an LOD of 0.01 mg/kg.

Half-lives ($t_{1/2}$) for 2,4-D in soil were calculated for each trial by regression analysis of the residue data. Verification techniques showed applications to be generally close to the targeted levels. Soil half-life values were calculated using only the residues found in the 0- to 15-cm soil layer. The lower layers were not included because the residues were generally low (0 or <10% of those in the 0-15 cm layer), and the rate of degradation has been shown to decrease with increasing soil depth.

Tables 15 and 16 show 2,4-D residues found at various samplings after the maximum number of applications in the 1993 and 1994 trials respectively. For the 2,4-D EHE treatments, the residues shown are the sum of 2,4-D plus the remaining 2,4-D EHE expressed as 2,4-D. Half-lives are also shown for each trial. These were determined from regression analyses using all sampling intervals, and from analyses using the highest residue value found as the initial value. When two half-life values are shown, the first was calculated from all the samplings and the second by taking the highest residue as the initial value.

Table 15. 2,4-D residues and half-lives in 1993 trials. All spray applications (Wilson *et al.*, 1997).

Type of trial, location	Compound applied	$t_{1/2, days}$	Residues, mg/kg, at days after application

			0	1	3	7	14	30	64
Bare soil	Amine	5.1-4.3	0.35[1]	0.38	0.36	0.53	0.05		
Wheat-CO	Ester	2.2	0.52[2]	0.48	0.35	0.16	0.01		
Bare soil	Amine	3.0	0.3	0.28	0.12	0.14	0.01[4]		
Wheat-NC	Ester	3.0-2.5	0.19	0.3	0.34[3]	0.05	0.01[4]		
Wheat	Amine	9.3-7.1	0.2	0.31	0.23	0.27	0.08		
CO	Ester	2.6-2.3	0.22	0.31	0.28[5]	0.04	<0.01		
Wheat	Amine	3.1-2.9	0.26	0.28	0.1[5]	0.17	<0.01[4]		
NC	Ester	6.1-6.5	0.12	0.12	0.17[3]	0.06[6]		<0.01	
Bare soil	Amine	2.5	0.89	0.38	0.16[3]	0.03[7]	0.013		
Turf-NC	Ester	1.7	0.62	0.63	0.36[3]	0.03[7]	<0.01		
Turf	Amine	2.3	0.88	0.57	0.13[3]	0.095[7]	<0.01		
NC	Ester	3.9	0.63	0.38	0.39[3]	0.04[7]	<0.01	<0.01[8]	
Pasture	Amine	10.7	0.44	0.38	0.21	0.15	0.09[4]	0.05	<0.01
TX	Ester	12.8	0.33	0.29	0.22	0.1	0.05[4]	0.05	<0.01

[1]Residues from amine applications are 2,4-D acid
[2]Residues from ester applications are the sum of 2,4-D acid and 2,4-D EHE expressed as 2,4-D ae
[3]Two days
[4]Fifteen days.
[5]Four days.
[6]Eight days.
[7]Five days.
[8]Thirty five days.
[9]Twenty five days.

Table 16. 2,4-D residues and half-lives in 1994 trials, spray and granule applications (Wilson *et al.*, 1997).

Type of trial	Compound applied	$t_{1/2, days}$	Residues, mg/kg, at days after application						
			0	1	3	7	14	30	60
Spray									
Bare soil	Amine	2.8	0.59[1]			0.32	0.05		
maize NE	Ester	4.1	0.52[2]			0.21	0.13	0.003	
Bare soil	Amine	16.1-15.9	0.47	0.75	0.73	0.5	0.44	0.07	0.01
maize OH	Ester	6.7-5.2	0.59	0.59	0.87	0.78	0.48	0.03	
Wheat	Amine	4.5	0.64	0.53	0.21	0.11	0.07	0.005	
ND	Ester	5.3	0.51	0.49	0.39	0.19	0.09	0.009	
Pasture	Amine	30.6-31.2	0.34	0.56	0.39	0.13	0.15	0.09[3]	0.04[4]
CA	Ester	25.6-27.5	0.29	0.34	0.15	0.09	0.08	0.08[3]	0.04[4]
Turf	Amine	7.5	0.12	0.09	0.08	0.21	0.02		
CA	Ester	8.5	0.15	0.13	0.04	0.09	0.02	0.01[5]	5
Bare soil	Amine	2.3-2.1	0.83	1.4	0.82	0.13	0.02		
turf CA	Ester	11.0	0.42	0.29	0.30	0.28	0.15		
Granular									
Turf ND	Amine	5.1-4.0	0.1	0.11	0.74	0.07	0.02	<0.01	
Bare soil	Amine	14.6-14.5	1.2	1.6	1.9	1.7	0.89	0.27	0.03[7]
turf ND									
Turf OH	Ester	296-84	0.18	0.74	0.28	0.25	0.19	0.07[6]	<0.01[4]
Bare soil	Ester	9.9	1.6	1.1	1.3	1.3	1.5	0.05[6]	0.01[4]
turf OH									

[1]Residues from amine applications are 2,4-D acid.
[2]Residues from ester applications are the sum of 2,4-D acid and 2,4-D EHE expressed as 2,4-D.
[3]Twenty nine days.
[4]Fifty eight days.
[5]Twenty days.
[6]Thirty one days.
[7]Sixty two days

Environmental fate in water/sediment systems

Aquatic degradation

Cohen (1991a) studied the aerobic aquatic degradation of uniformly ring-labelled [^{14}C]2,4-D at a concentration of 4.6 mg/kg in a mixture of sieved (2-mm), unsterilized Louisiana rice paddy sediment (clay soil) and water (320 g sediment, 534 g water, 854 g total). The untreated mixture was pre-incubated under aerobic aquatic conditions in darkness at 25°C for 218 days to activate microbes, then [^{14}C]2,4-D in acetonitrile was mixed thoroughly with the sediment/water in the incubation flask and aerobically incubated in darkness at 25°C for 30 days. The soil was continuously purged with humidified air at 30 ml/minute to flush any volatile compounds formed into a series of trapping solutions (ethylene glycol, 1 *M* sulfuric acid and 5% sodium hydroxide).

Samples of the test system were taken immediately after treatment (day 0) and at 2, 5, 12, 20, 27 and 30 days after treatment. Sediment/water samples were separated by centrifugation and aliquots of sediment were combusted and the ^{14}C determined by LSC. The remaining sediment samples were extracted with 1.5 *M* phosphoric acid mixed with ethyl ether, washed with water, and re-extracted with 1 *N* sodium hydroxide solution. ^{14}C was measured in aliquots of all extracts and in duplicate samples of the supernatant and trapping solutions by LSC.

The sediment extracts and the water supernatant were analysed by HPLC with both UV (280 nm) and radio detection in series. Radioactive flow detection allowed identification of 2,4-D and its aerobic degradation products at a level of 0.01 mg/kg (limit of detection 0.005 mg/kg).

Analysis of the sediment/water samples showed that the half-life of 2,4-D acid was 15 days under aerobic aquatic conditions.

The degradation product chlorohydroquinone reached a maximum concentration of 17% (0.78 mg/kg) of the initial radioactivity on day 27 and decreased to 11% (0.52 mg/kg) by day 30. A minor product, 2,4-dichlorophenol, accounted for 4.9% (0.23 mg/kg) and CO_2 for 16% (0.74 mg/kg) of the initial ^{14}C by day 30.

The recovery of ^{14}C during the study (soil extractable and unextractable + water-soluble + cumulative volatiles) ranged from 69 to 100% of the initial radioactivity.

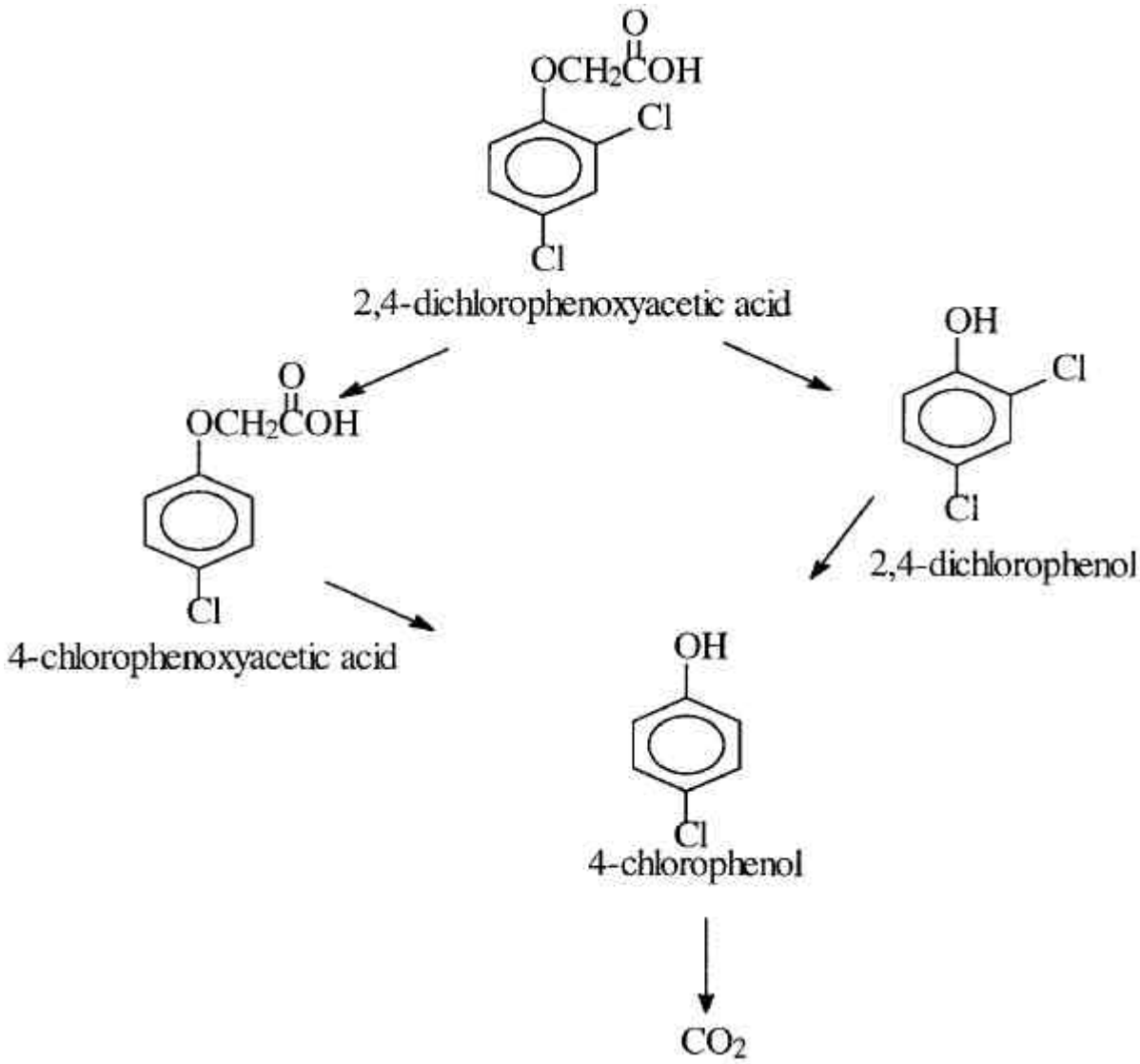
Concha and Shepler (1993a) studied aerobic aquatic degradation of [^{14}C]2,4-D in pond sediment and water samples from Henry County, Illinois with an application rate of 5 mg/kg for up to 46 days, and incubation at 25°C. The aqueous phase was monitored at each sampling for pH (7 to 8) and dissolved oxygen content (2.7 to 6.75 mg/kg).

[^{14}C]2,4-D acid was degraded slowly in the first 25 days and represented $\leq$75% of the applied dose at 25 days. It decreased rapidly n the next 10 days, and at 46 days it represented 0.5% of the applied radiocarbon. Its half-life was 4.5 days.

The main degradation product was CO_2 (64% of the applied radiocarbon at 46 days). 2,4-DCP, 4-CPA and 4-chlorophenol were present in both the water and sediment extracts. 2,4-DCP accounted for 1.1% of the applied ^{14}C at day 35 and decreased to 0.1% at day 46, and 4-CPA rose to 1.1% at day 14 and decreased to untraceable amounts. 4-chlorophenol represented 1.4% of the applied radiocarbon after 20 days and decreased slowly to zero by day 46. An unknown product was observed in the water phase of the samples after 35 days (1.1% of the applied dose); it was unstable when stored frozen or refrigerated and was converted to CO_2 and other volatile products upon acidification of the water-phase. No organic volatiles were detected above 0.1%. The unextractable residue increased with exposure time and constituted about 16% of the applied dose at day 46.

Radiocarbon recoveries averaged 93 ± 7%. During the lag time (25 days) most of the radiocarbon (>64%) was found in the water, and approximately 10 to 14% was extracted from the sediment in an alkaline solvent and about 4% in acidic acetone. After the lag time the radiocarbon distribution shifted dramatically as 2,4-D acid was degraded. At day 46 3% of the applied dose was recovered in the water phase, with 1.0% and 0.6% extracted in base and acidic solvents respectively. The overall material balance dropped slightly at day 35 but increased at day 46. This decrease is attributed to the rapid, massive production of CO_2 after the lag period that may not have been trapped efficiently which the trapping solutions were being changed. A separate sample was incubated and left unopened for 39 days, and higher recovery (92%) confirmed that the loss of radiocarbon was owing to the loss of untrapped CO_2 in the headspace.

Figure 6. Degradation pathways of 2,4-D in an aerobic pond sediment/water system (Concha and Shepler, 1993a).



Concha and Shepler (1994b) studied the anaerobic aquatic degradation of [^{14}C]2,4-D (4.9 mg/kg) in raw pond sediment and water in Henry County, Illinois for one year. Samples were incubated at 25°C throughout the study and the aqueous phase was monitored at each sampling for pH (7.6-9.6). CLICK HERE for continue