

## 2,4-D (Pesticides)

### Summary

#### Food Safety Commission of Japan

Food Safety Commission of Japan (FSCJ) conducted a risk assessment of 2,4-D<sup>1)</sup> (CAS No. 94-75-7), a phenoxy herbicide, based on results from various studies. Major adverse effects of 2,4-D observed were suppressed body weight, renal tubular degeneration, hypertrophy of hepatocytes, reduced weight of testis and retinal degeneration in rats. No adverse effects were detected in carcinogenicity, reproductive toxicity, teratogenicity and genotoxicity relevant to human health. The relevant substance to the residue definition for dietary risk assessment was identified as 2,4-D and metabolite C<sup>2)</sup> in agricultural products and 2,4-D (parent compound only) in livestock products. The lowest no-observed-effect level (NOAEL) obtained from all the studies was 0.99 mg/kg bw/day in a two-year combined chronic toxicity/carcinogenicity study (the 1<sup>st</sup> study in **Table 1**) in rats. FSCJ specified an acceptable daily intake (ADI) of 0.0099 mg/kg bw/day by applying a safety factor of 100 to the NOAEL. The lowest NOAEL for adverse effects likely to be elicited by a single oral administration of 2,4-D was 15 mg/kg bw/day obtained from the acute neurotoxicity study in rats. Consequently, FSCJ specified an acute reference dose (ARfD) of 0.15 mg/kg bw by applying a safety factor of 100 to the NOAEL.

#### Conclusion in Brief

Food Safety Commission of Japan (FSCJ) conducted a risk assessment of 2,4-D<sup>1)</sup> (CAS No.94-75-7), a phenoxy herbicide, based on results from various studies.

The data used in the assessment include the fate in animals (rats, mice, goats, chickens and humans), fate in plants (paddy rice, wheat and others), residues in crops, acute neurotoxicity (rats), subacute toxicity (rats, mice and dogs), chronic toxicity (dogs), combined chronic toxicity/carcinogenicity (rats), carcinogenicity (mice), two-generation reproductive toxicity (rats), developmental toxicity (rats and rabbits), genotoxicity, and also on their mechanisms.

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Based on the results from various studies, the relevant

substance to the residue definition for dietary risk assessment was identified as 2,4-D and metabolite C<sup>2)</sup> in agricultural products and 2,4-D (parent compound only) in livestock products.

The lowest no-observed-effect level (NOAEL) obtained from all the studies was 0.99 mg/kg bw/day in a two-year combined chronic toxicity/carcinogenicity study (the 1<sup>st</sup> study in **Table 1**) in rats. FSCJ specified an acceptable daily intake (ADI) of 0.0099 mg/kg bw/day by applying a safety factor of 100 to the NOAEL.

The lowest NOAEL for adverse effects likely to be elicited by a single oral administration of 2,4-D was 15 mg/kg bw/day obtained from the acute neurotoxicity study in rats. Consequently, FSCJ specified an acute reference dose (ARfD) of 0.15 mg/kg bw by applying a safety factor of 100 to the NOAEL.

<sup>1)</sup> 2,4-dichlorophenoxy acetic acid

<sup>2)</sup> 2,4-dichlorophenol

**Table 1.** Levels relevant to toxicological evaluation of 2,4-D

Species	Study	Dose (mg/kg bw/day)	NOAEL (mg/kg bw/day) and critical endpoints <sup>1)</sup>
Rat	90-day subacute toxicity study (the 1 <sup>st</sup> study)	0, 1, 5, 15, 45	M/F: 1 M/F: Renal lesion
	90-day subacute toxicity study (the 2 <sup>nd</sup> study)	0, 1, 15, 100, 300 M: 0, 0.93, 14.0, 93.9, 278 F: 0, 0.96, 14.4, 96.2, 293	M: 14.0 F: 14.4 M/F: Suppressed body weight, etc.
	90-day subacute toxicity study (the 3 <sup>rd</sup> study)	0, 15, 60, 100, 150	M/F: 15 M/F: Renal tubular lesion, etc.
	Two-year combined chronic toxicity/carcinogenicity study (the 1 <sup>st</sup> study)	0, 1, 5, 15, 45 M: 0, 0.99, 4.95, 14.8, 44.5 F: 0, 0.99, 4.96, 14.9, 44.7	M/F: 0.99 M/F: Brown pigmentation of renal tubule, etc. (Not carcinogenic)
	Two-year combined chronic toxicity/carcinogenicity study (the 2 <sup>nd</sup> study)	0, 5, 75, 150 M: 0, 4.77, 73.2, 145 F: 0, 4.89, 73.1, 144	M: 4.77 F: 4.89 M/F: Increase in ALP, etc. (Not carcinogenic)
	One-year chronic neurotoxicity study	0, 5, 75, 150 M: 0, 4.77, 73.2, 145 F: 0, 4.89, 73.1, 144	M: 4.77 F: 4.89 M/F: Suppressed body weight (No chronic neurotoxicity)
	Two-generation reproductive toxicity study	0, 5, 20, 80 PM: 0, 5.0, 20.1, 79.8 PF: 0, 5.0, 19.9, 78.5 F <sub>1</sub> M: 0, 5.0, 19.2 F <sub>1</sub> F: 0, 5.0, 20.2	Parent PM: 5.0 PF: 19.9 F <sub>1</sub> M: 5.0 F <sub>1</sub> F: 20.2 Offspring PM: 5.0 PF: 5.0 F <sub>1</sub> M: 5.0 F <sub>1</sub> F: 5.0 Parent M: Localized tubular degeneration in renal medulla F: Suppressed body weight, etc. Offspring: Lower body weight (No adverse effect on fertility)
	Extended one-generation reproductive toxicity study	0, 100, 300, 600(F)/800(M) ppm	P generation M: 16.6 (300 ppm) F: 40.2 (600 ppm) M: Increased absolute/relative renal weight, etc. F: No toxicity F <sub>1</sub> generation M: 20.9 (300 ppm) F: 23.3 (300 ppm) M/F: Suppressed body weight (During lactation period) F <sub>1</sub> generation F(Adult): Proximal tubular degeneration in kidney (No adverse effects on fertility, developmental neurotoxicity, and developmental immunotoxicity)
	Developmental toxicity study (the 1 <sup>st</sup> study)	0, 12.5, 25, 50, 75, 88	Maternal: 88 Fetus: 25 Maternal: No toxicity Fetus: Lower body weight, increased skeletal variations (lumbar rib, wavy rib), etc. (Not teratogenic)

**Table 1.** Levels relevant to toxicological evaluation of 2,4-D (continued)

Species	Study	Dose (mg/kg bw/day)	NOAEL (mg/kg bw/day) and critical endpoints <sup>1)</sup>
Rat	Developmental toxicity study (the 2 <sup>nd</sup> study)	0, 8, 25, 75	Maternal: 25 Fetus: 25 Maternal: Suppressed body weight Fetus: Increased skeletal variations (Not teratogenic)
Mouse	90-day subacute toxicity study (the 1 <sup>st</sup> study)	0, 5, 15, 45, 90	- M/F: Renal lesion
	90-day subacute toxicity study (the 2 <sup>nd</sup> study)	0, 1.0, 15.0, 100, 300 M: 0, 0.98, 14.7, 98.2, 293 F: 0, 0.99, 14.8, 98.9, 296	M: 14.7 F: 14.8 M: Decrease in T <sub>4</sub> F: Decrease in Glu, etc.
	Two-year carcinogenicity study (the 1 <sup>st</sup> study)	0, 1, 15, 45 M: 0, 0.98, 14.9, 44.8 F: 0, 1.00, 14.9, 44.8	M: 0.98 F: 14.9 M: Homogenous change of renal tubular epithelium F: Increased absolute/relative renal weight (Not carcinogenic)
	Two-year carcinogenicity study (the 2 <sup>nd</sup> study)	F: 0, 5, 150, 300 F: 0, 5.01, 150, 310	F: 5.01 F: Increased absolute/relative renal weight, etc. (Not carcinogenic)
	Two-year carcinogenicity study (the 3 <sup>rd</sup> study)	M: 0, 5, 62.5, 125 M: 0, 5.0, 61.9, 129	M: 5.0 M: Proximal tubular degeneration/regeneration in kidney, etc. (Not carcinogenic)
Rabbit	Developmental toxicity study	0, 10, 30, 90	Maternal: 30 Fetus: 90 Maternal: Suppressed body weight, etc. Fetus: No toxicity (Not teratogenic)
Dog	90-day subacute toxicity study (the 1 <sup>st</sup> study)	0, 0.3, 1, 3, 10	M: 1 F: 3 M/F: Change of renal convoluted proximal tubule, etc.
	90-day subacute toxicity study (the 2 <sup>nd</sup> study)	0, 0.5, 1, 3.75, 7.5 M: 0, 0.5, 1.0, 3.8, 7.8 F: 0, 0.5, 1.0, 3.8, 7.7	M/F: 1.0 M/F: Suppressed body weight, etc.
	One-year chronic toxicity study	0, 1, 5, 10/7.5* M: 0, 1.0, 5.2, 8.2 F: 0, 1.0, 5.0, 7.9 *: In the 8 <sup>th</sup> week after administration, the dose was cut to 7.5 mg/kg bw/day	M/F: 1.0 M/F: Suppressed body weight, etc.
ADI (cRfD)			NOAEL: 0.99 SF: 100 ADI: 0.0099
The critical study for setting ADI			Two-year combined chronic toxicity/carcinogenicity study in rats (the 1 <sup>st</sup> study)

M, Male; F, Female; M/F, both sexes; PM, Male in P (Parent) generation; PF, Female in P generation; F<sub>1</sub>M, Male in F<sub>1</sub> generation; F<sub>1</sub>F, Female in F<sub>1</sub> generation; -, NOAEL could not be specified; ADI, Acceptable daily intake; cRfD, Chronic reference dose; SF, Safety factor; UF, Uncertainty factor; NOAEL, No-observed-adverse-effect level; NOEL, No-observed-effect level; -, NOAEL could not be specified

<sup>1)</sup> The adverse effect observed at the lowest-observed-adverse-effect level (LOAEL)

**Table 2.** Potential adverse effects of a single oral administration of 2,4-D

Species	Study	Dose (mg/kg bw/day)	NOAEL (mg/kg bw/day) and critical endpoints <sup>1)</sup>
Rat	Acute toxicity study	0, 197(M), 250, 318, 403, 512, 650, 826	M/F: - M/F: Hypoactivity, abnormal gait (1 hour after the treatment)
	Acute neurotoxicity study	0, 15, 75, 250	M: 75 F: 15 M: Motor coordination ataxia, abnormal gait, decreased locomotor activity (5–6 hours after the treatment) F: Abnormal gait (5–6 hours after the treatment)
Mouse	General pharmacology study (General state)	0, 30, 100, 300	30 Abnormal/ataxic gait (0.5–5 hours after the treatment)
	Acute toxicity study	0, 200, 264, 348, 460, 670, 801	M/F: - M/F: Hypoactivity, abnormal gait (1 hour after the treatment)
Rabbit	General pharmacology study (General state)	M:0, 30, 100, 300	M: 100 Abnormal gait, decreased locomotor activity (3–4 hours after the treatment)
	General pharmacology study (Body temperature)	M:0, 30, 100, 300	M: 100 Elevated body temperature (2–3 hours after the treatment)
ARfD			NOAEL: 15 SF: 100 ARfD: 0.15
The critical study for setting ARfD			Acute neurotoxicity study in rats

ARfD, Acute reference dose; SF, Safety factor; NOAEL, No-observed-adverse-effect level; -, NOAEL or LOAEL could not be specified

<sup>1)</sup> The adverse effect observed at LOAEL

**Table 3.** Levels relevant to toxicological evaluation of 2,4-D

Species	Study	Test substances	Dose (mg/kg bw/day)	NOAEL (mg/kg bw/day) and critical endpoints <sup>1)</sup>
Rat	90-day subacute toxicity study	DEA salt	0, 1.5, 27, 150, 440 [0, 1, 18, 100, 300]	27 [18] Elevated mortality rate, etc.
		DMA salt	0, 1.2, 18, 120, 360 [0, 1, 15, 100, 300]	18 [15] Suppressed body weight, etc.
		IPA salt	0, 1, 19, 130, 380 [0, 1, 15, 100, 300]	19 [15] Suppressed body weight, etc.
		TIPA salt	0, 2, 28, 190, 560 [0, 1, 15, 100, 300]	28 [15] Histopathological change in kidney, etc.
		BEH ester	0, 1.5, 22, 140, 440 [0, 1, 15, 100, 300]	22 [15] Suppressed body weight, etc.
		EH ester	0, 1.5, 23, 150, 450 [0, 1, 15, 100, 300]	23 [15] Suppressed body weight, etc.
	Developmental toxicity study	DEA salt	0, 15, 75, 150 [0, 11, 55, 110]	Maternal: 15 [11] Fetus: 15 [11] Maternal: Suppressed body weight Fetus: Increased skeletal variations (Not teratogenic)
		DMA salt	[0, 12, 50, 100]	Maternal: [12] Fetus: [50] Maternal: Suppressed body weight Fetus: Lower body weight, etc. (Not teratogenic)
		IPA salt	0, 22, 65, 190 [0, 9, 25, 74]	Maternal: 65 [25] Fetus: 190 [74] Maternal: Suppressed body weight, etc. Fetus: No toxicity (Not teratogenic)
		TIPA salt	0, 32, 100, 320 [0, 12, 37, 120]	Maternal: 100 [37] Fetus: 32 [12] Maternal: Death, etc. Fetus: Increase in skeletal variations
		BEH ester	0, 25, 75, 180 [0, 17, 50, 120]	Maternal: 75 [50] Fetus: 75 [50] Maternal: Suppressed body weight Fetus: Delayed ossification (Not teratogenic)
		EH ester	[0, 10, 30, 90]	Maternal: [30] Fetus: [30] Maternal: Suppressed body weight, etc. Fetus: Delayed ossification (Not teratogenic)

**Table 3.** Levels relevant to toxicological evaluation of 2,4-D (continued)

Species	Study	Test substances	Dose (mg/kg bw/day)	NOAEL (mg/kg bw/day) and critical endpoints <sup>1)</sup>
Rabbit	Developmental toxicity study	DEA salt	[0, 15, 30, 60]	Maternal: [15] Fetus: [30] Maternal: Suppressed body weight, etc. Fetus: Increased skeletal variations (Not teratogenic)
		DMA salt	[0, 10, 30, 90]	Maternal: [30] Fetus: [90] Maternal: Reduced motor activity, etc. Fetus: No toxicity (Not teratogenic)
		IPA salt	0, 13, 38, 95 [0, 10, 30, 75]	Maternal: 13 [10] Fetus: 95 [75] Maternal: Death, etc. Fetus: No toxicity (Not teratogenic)
		TIPA salt	0, 19, 56, 140 [0, 10, 30, 75]	Maternal: 19 [10] Fetus: 140 [75] Maternal: Death, etc. Fetus: No toxicity (Not teratogenic)
		BEH ester	0, 15, 45, 110 [0, 10, 30, 75]	Maternal: 15 [10] Fetus: 110 [75] Maternal: Death, etc. Fetus: No toxicity (Not teratogenic)
		EH ester	[0, 10, 30, 75]	Maternal: [30] Fetus: [75] Maternal: Death, etc. Fetus: No toxicity (Not teratogenic)
Dog	90-day subacute toxicity study	DMA salt	[0, 1, 3.8, 7.5]	M/F: [1] M/F: Suppressed body weight, etc.
		EH ester	[0, 1, 3.8, 7.5]	M/F: [1] M/F: Suppressed body weight, etc.

The values given in parentheses [ ] are the corresponding values in acid

DEA, dethanolamine; DMA, dimethylamine; IPA, Isopropylamine; TIPA, Triisopropanolamine; BEH, butoxyethyl; EH, ethylhexyl

<sup>1)</sup> The adverse effect observed at LOAEL

**Table 4.** Potential adverse effects of a single oral administration of 2,4-D

Test substances	Species	Study	Dose (mg/kg bw/day)	NOAEL (mg/kg bw/day) and critical endpoints <sup>1)</sup>
2,4-D ethyl	Rat	Acute toxicity study	250, 350, 500	M/F: - M/F: Hypoactivity, etc. (1 hour after the treatment)
	Mouse	Acute toxicity study	0, 125, 250, 290(M), 335(M), 375, 500	M/F: - M/F: Hypoactivity, fece/soil (1 hour after the treatment)
Na salt	Rat	Acute toxicity study	250, 500, 1 000, 2 000	M/F: - M/F: Ataxia, hypoactivity, etc. (1 hour after the treatment)
	Mouse	Acute toxicity study	125, 250, 375, 500, 1 000	M: - F: 125 M/F: Hypoactivity, etc. (1 hour after the treatment)
DMA salt	Rat	Acute toxicity study	500, 710, 1 000	M/F: - M/F: Ataxia, hypoactivity, etc. (1 hour after the treatment)
	Mouse	Acute toxicity study	250, 500, 1 000, 2 000, 4 000	M/F: - M/F: Hypoactivity, ataxia, etc. (1 hour after the treatment)
IPA salt	Rat	Acute toxicity study	500, 750, 1 000, 5 000	M/F: - M/F: Stiffness in handling (1 day after the treatment)
DEA salt	Rat	Developmental toxicity study	0, 15, 75, 150 [0, 11, 55, 110]	Maternal: 15 [11] Maternal: Suppressed body weight (6–9 days after GD)
TIPA salt	Rat	Developmental toxicity study	0, 32, 100, 320 [0, 12, 37, 120]	Maternal: 100 [37] Embryo/fetus: 32 [12] Maternal: Death, rigid limbs, salivation, suppressed body weight (During the treatment) Embryo/fetus: Increased skeletal variation (wavy rib)
BEH ester	Rat	Developmental toxicity study	0, 25, 75, 180 [0, 17, 50, 120]	Maternal: 75 [50] Maternal: Suppressed body weight (6–9 days after GD)
EH ester	Rat	Developmental toxicity study	[0, 10, 30, 90]	Maternal: [30] Maternal: Suppressed body weight (6–9 days after GD)

The values given in parentheses [ ] are the corresponding values in acid  
GD, gestation day; Na salt, sodium salt; other abbreviation are described in **Table3**.

<sup>1)</sup> The adverse effect observed at LOAEL