Risk Assessment Report on Amitraz

(Pesticide and Veterinary Medicines)

Food Safety Commission of Japan (FSCJ)

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Summary

We conducted a risk assessment of an insecticide (milticide), Amitraz (IUPAC: *N'*-(2,4-dimethylphenyl)-*N*-[[(2,4-dimethylphenyl)imino]methyl]-*N*- methylmethane imidamide) by evaluating various assessment reports and other materials (Directory of Agricultural Chemicals, JMPR Report, U.S. EPA Report, Health Canada Report, APVMA Report, and summary of application for import of veterinary medicine).

The results shown in such assessment reports and other materials are derived from tests on metabolic fate in animals (mice, rats, dairy cows, calves, pigs, dogs, honeybees) and humans, plants (apples, lemons, pears, cucumbers, and green beans), soil and water, as well as testing of soil residues and crop residues, acute toxicity tests (on rats, mice, guinea pigs, rabbits, dogs, and baboons), subchronic toxicity tests (on rats, mice, rabbits, and dogs), chronic toxicity tests (on dogs), chronic toxicity/carcinogenicity tests (on rats and mice), carcinogenicity tests (on mice), three-generation productivity tests (on rats), developmental toxicology tests (on rats and rabbits), genotoxicity tests, and others.

The results of toxicity tests indicated a mild level of suppression of the central nervous system, with dogs showing the highest sensitivity. No concerning teratogenicity or genotoxicity in living organisms was recognized. In carcinogenicity tests, frequencies of lymphoreticular tumors and liver tumors increased in female mice, but these increases occurred only at high doses where toxicity was clearly shown, and no genotoxicity was observed. Hence, the developmental mechanism is considered non-genetic and it was considered possible to set a threshold for this formulation in this assessment.

The lowest value among no-observed-adverse-effect levels (NOAELs) was 0.25 mg/kg bw/day, which was obtained in the two-year chronic toxicity test in dogs. Based on this figure and the safety factor of 100, the acceptable daily intake (ADI) for amitraz is established as 0.0025 mg/kg bw/day.

Risk Assessment

The health risk assessment on amitraz was conducted using the materials listed in the Reference.

In animal metabolism tests, amitraz was rapidly metabolized in animal bodies and excreted when orally administered. The main excretion was urinal (approximately 80% TAR) and the rest was excreted in feces. The main metabolites were G, H, and B. In plant metabolism tests, the level of the substance found in fruits was low. The main metabolites in plant metabolism tests were metabolite B and C. Residue tests confirmed that the level of amitraz would not exceed the value in the provisionally set standards when administered to honeybees as a veterinary medicine.

The residual level of amitraz and its metabolite B was examined. The highest level of residual amitraz was 1.21 mg/kg, found in the skin of summer orange (*Citrus natsudaidai*) harvested on the 21st day after the last application. The highest level of residual metabolite B was 1.61 mg/kg, found in the skin of mandarin orange harvested on the 14th day and 28th day after the last application.

Based on the various studies to test fate and residual properties, amitraz and its metabolite B were determined as the substances to be evaluated for exposure in agricultural products.

Various studies were conducted to test the toxicological properties of amitraz. A slight level of suppression of the central nervous system was found, with dogs being the most sensitive. No teratogenicity or genotoxicity of concern to living bodies was found. In a carcinogenicity study, frequencies of tumors on lymph/reticulum cells and hepatophyma increased in female mice, but the increase was seen only with doses high enough to clearly show toxicity and no genotoxicity was observed. Therefore, the developmental mechanism was considered to be a non-genotoxicity mechanism and it was concluded that the setting of a threshold would be possible. It is also noted that while the assessment was conducted with a limited set of data obtained from the studies conducted before the implementation of GLP regulations, the use of such data was considered feasible to carry out the assessment.

NOAELs and other values referred to in the assessment are shown in Table A, which were determined in each study and report.

The lowest value among NOAELs was 0.25 mg/kg bw/day, which was obtained in a 2-year chronic study using dogs. Based on this figure and the safety factor of 100, the Food Safety Commission has established the ADI for amitraz as 0.0025 mg/kg bw/day.

ADI	0.0025 mg/kg bw/day
(Referred data to set ADI)	Chronic toxicity study
(Animal species)	Dog
(Test period)	2 years
(Administration method)	By gavage
(NOAEL)	0.25 mg/kg bw/day
(Safety factor)	100

The level of exposure is to be confirmed when provisional standards are reviewed based on the results of this evaluation.

Table A. Comparison of NOAELs Obtained in Various Tests

Animal species	Test	Dose (mg/kg bw/day)	NOAEL (mg/kg bw/day)[1)]				
			Directory of Agricultural Chemicals	JMPR	U.S.A	Canada	Australia
Rat	90-day subchronic toxicity test	0, 3, 12	3 Suppression of body weight gain, etc.	3 Suppression of body weight gain, etc.	3 Suppression of body weight gain, etc.		
	21-day repeated inhalation toxicity test	0, 0.01, 0.1, 1.0 mg/L	0.01 mg/L Suppression of body weight gain, aggressive behaviors, etc.	– Suppression of body weight gain, aggressive behaviors, etc.	0.01 mg/L Suppression of body weight gain, aggressive behaviors, etc.		
	2-year chronic toxicity/ carcinogenicity test	0, 15, 50, 200 ppm Male: 0, 0.77, 2.50, 10.2 Female: 0, 0.97, 3.13, 12.6	Male:2.50 Female: 0.97 Excitation and aggressiveness (no carcinogenicity observed)	2.5 Excitation and aggressiveness, suppression of body weight gain, etc. (no carcinogenicity observed)	Male:2.5 Female: 0.97 Excitation and aggressiveness, suppression of body weight gain, etc. (no carcinogenicity observed)		– Oversensitivity and aggression
	Three-generation reproductive toxicity test	0, 15, 50, 200 ppm Male: 0, 1.29, 4.36, 16.4 Female: 0, 1.58, 5.09, 20.1	Male: 1.29 Female: 1.58 Increase in mortality, etc.	Parent animals: 4.4 Reproductive toxicity: 1.3 Increase in mortality, etc.	Parent animals: Male: 4.36 Female: 5.09 Offspring: Male: 1.29 Female: 1.58 Increase in mortality, etc. (No adverse effects on reproduction)		1.29 Increase in mortality, etc.

Rat	Developmental toxicity test 1	0, 1, 3, 12	Dam: 3 Offspring: 3 Dam: Suppression of body weight gain Offspring: Lower body weight (No teratogenicity was found)	Dam: 12 Offspring: 3 Dam: No toxicity found Offspring: Lower body weight (No teratogenicity was found)	Dam: 3 Offspring: 12 Dam: Suppression of body weight gain Offspring: no toxicity found (No teratogenicity was found)		
	Developmental toxicity test 2	0, 7.5, 15, 30		Dam and offspring: 7.5 Dam: Suppression of body weight gain Offspring: Expansion of urinal duct and renal pelvis	Dam: 7.5 Offspring: 30 Dam: Suppression of body weight gain, etc. Offspring: No significant difference in the increase in expansion of urinal duct and renal pelvis		

Mouse	90-day subchronic toxicity test	0, 100, 200, 400, 600, 800 ppm Male: 0, 12.6, 25.5, 53.4, 96.2, 108 Female: 0, 17.2, 34.5, 68.2, 112, 151	Male: 25.5 Female: 17.2 Suppression of body weight gain, increase in aggressive behavior (male), etc.	17 Suppression of body weight gain, increase in aggressive behavior (male), etc.			– Suppression of body weight gain, increase in aggressive behavior (male)
	2-year chronic toxicity/ carcinogenicity test	0, 7, 25, 100, 400 ppm Male: 0, 0.93, 3.36, 13.6, 60.4 Female: 0, 0.90, 3.16, 12.8, 56.7	Male: 3.36 Female: 0.90 Decline of locomotor activities, suppression of body weight gain, etc. (No carcinogenicity found)				
	18-month carcinogenicity test	0, 25, 100, 400 ppm Male: 0, 2.79, 12.5, 66.5 Female: 0, 4.11, 16.3, 84.5	Male: 2.79 Female: 4.11 Suppression of body weight gain, etc. Increased frequency of tumors on lymph/reticulum cells in females at 400 ppm	15 Increase in hepatocellular tumors (males and females), increased frequency of lymph/reticulum cells (females) at 400 ppm.			– Increased frequency of lymph/reticulum cells at 400 ppm.
	2-year carcinogenicity test	0, 25, 100, 400 ppm Male: 0, 2.3, 9.6, 44.7 Female: 0, 2.6, 10.8, 50.1	Male: – Female: – Male: hyperkeratosis of stomach, etc. Female: hypertrophic nodule formation, etc. Slight increased occurrence of hepatic tumors in females at 400 ppm.	Long-term toxicity: 2.3 Carcinogenicity: 11 Suppression of body weight gain, decline of M/E ratio, aggressive behaviors, etc. Slight increase in hepatic tumors in females at 400 ppm.	– Dose-related increase in lung adenomatosis (male) and hepatic adenoma and cancer (male)		2.2 Aggressive behaviors Increase in hepatic tumors and cancer

Rabbit	Developmental toxicity test 1	0, 1, 5, 25	Dam: 5 Offspring: 25 Dam: Suppression of body weight gain, miscarriage, etc. Offspring: No toxicological findings (No teratogenicity was found)	Dam: 25 Offspring: 5 Dam: No toxicological findings Offspring: Miscarriage, decrease in litter size, etc. (No teratogenicity was found)	Dam: 5 Offspring: 5 Dam: Decrease in body weight, miscarriage Offspring: Decline of litter size, decrease in average body weight of fetuses, etc. (No teratogenicity was found)		– Decline of litter size
Dog	90-day subchronic toxicity test	0, 0.25, 1.0, 4.0	Male and female: 0.25 Suppression of central nervous system, etc.	0.25 Suppression of central nervous system, etc.	0.25 Suppression of central nervous system, etc.		0.25 Increase in blood glucose
	2-year chronic toxicity test	0, 0.1, 0.25, 1.0	Male and female: 0.25 Slight level of suppression of central nervous system	0.25 Slight suppression of central nervous system	0.25 Slight suppression of central nervous system		0.25 Increase in blood glucose
Human	Double-blind test	0, 0.0625, 0.125	0.125 No toxicity findings	0.13 No toxicity findings		0.125 No toxicity findings	
ADI (cRfD)			NOAEL: 0.25 SF: 100 AD: 0.0025	NOAEL: 1.3 SF: 100 ADI: 0.01	NOAEL: 0.25 UF: 1000 cRfD: 0.00025	NOAEL: 0.125 SF: 10 ADI: 0.0125	NOAEL: 0.25 SF: 100 ADI: 0.002
Referred data for ADI			2-year chronic toxicity test using dogs	Three-generation reproductive test in rats	2-year chronic toxicity test using dogs	Double-blind test in humans	2-year chronic toxicity test using dogs

Cell with diagonal line: No test result reported.
Minus sign (-): Setting of NOAEL was not possible (or NOAEL was not reported).
NOAEL: no-observed-adverse-effect level; SF: safety factor; UF: uncertainty factor; ADI: acceptable daily intake; cRfD: chronic reference dose
1): Major toxicity findings observed at the minimum toxic level are shown along with the NOAEL.
2): EMEA follows the JMPR regulations.