HAZARD ASSESSMENT REPORT

N, *N*-Dimethylformamide

CAS No. 68-12-2

Chemicals Evaluation and Research Institute (CERI), Japan

This report was prepared by CERI in collaboration with National Institute of Technology and Evaluation (NITE) under the sponsorship of New Energy and Industrial Technology Development Organization (NEDO).

Preface to the English Version of the Hazard Assessment Reports

For six years from April 2001 to March 2007, Chemicals Evaluation and Research Institute (CERI/Japan) was engaged in a project named “Chemical Risk Assessment and Development of Risk Assessment Methods” under "Comprehensive Chemical Substance Assessment and Management Program" funded by New Energy and Industrial Technology Development Organization (NEDO/Japan). Under this project, about 150 chemical substances were selected among those designated as Class-I Chemicals in the Law for Pollutant Release and Transfer Register and Promotion of Chemical Management (hereafter PRTR Law)[1]. The selection criteria of these chemicals were their priorities for risk assessment based on their production levels and environmental/human health concerns.

CERI developed the hazard assessment reports of these selected chemical substances based on the review and evaluation of the environmental and human health hazard data obtained from the existing evaluation documents released by the regulatory agencies and international organizations as well as those from the published scientific literatures. The data review and compilation of the reports were conducted according to the guidelines[2] and the guidance manual[2] developed for this project. The proposed hazard assessment reports by CERI were reviewed by the experts in the relevant scientific fields from both inside and outside this project for accuracy, relevance and completeness. The final reports were published in Japanese after going through the deliberation by the “Council on Chemical Substances” under the Ministry of Economy, Trade and Industry (METI/Japan), which is responsible for regulation of chemical substances in Japan.

This project was the first attempt in Japan to develop comprehensive hazard assessments of chemical substances for application in risk assessment. In order to share the outcomes of the project globally, CERI independently selected the following seven chemical substances and developed the English version of the hazard assessment reports:

(1) Acetaldehyde
(2) Chlorobenzene
(3) Hydrazine
(4) *N*, *N*-Dimethylformamide
(5) Poly(oxyethylene) nonylphenyl ether
(6) 3,3’-Dichloro-4,4’-diaminodiphenylmethane
(7) Dimethyl 2,2-dichlorovinyl phosphate (Dichlorvos)

We hope that the hazard assessment reports from our project contribute to the risk assessment and management of chemical substances globally, and appreciate your feedback.

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[1] Details of the PRTR Law, the list of designated chemical substances, and release data in Japan are available on Internet at: http://www.prtr.nite.go.jp/index-e.html.

[2] Guidelines and the guidance manual in Japanese are available on Internet at: http://www.safe.nite.go.jp/risk/riskhykdl01.html. Also, the initial risk assessment reports in Japanese developed in this project which include calculations of margin of exposure based on the result of hazard assessment and exposure assessment, are available on Internet at: http://www.safe.nite.go.jp/risk/riskhykdl01.html.

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Summary

N, *N*-Dimethylformamide is a colorless or slightly yellow liquid with a boiling point of 153°C and a vapor pressure of 380 Pa at 20°C. It is freely soluble in water and soluble in alcohols, acetone and benzene. *N*, *N*-Dimethylformamide is used as solvent, catalyst and gas absorbent. Sums of domestic production and import levels of *N*, *N*-dimethylformamide were 63,043 and 57,724 tons in F.Y. of 2001 and 2002, respectively.

Considering the uses of *N*, *N*-dimethylformamide and based on the annual emission data for F.Y. 2001 in Japan (the 2001 PRTR data), the main release route is considered through emissions in the use process of *N*, *N*-dimethylformamide and products containing *N*, *N*-dimethylformamide. As the scenario of *N*, *N*-dimethylformamide releases in Japan, it is estimated that 24,951 tons of *N*, *N*-dimethylformamide was released annually in air and 1,137 tons in water.

N, *N*-Dimethylformamide released into the aquatic environment is eliminated mainly by biodegradation. It is considered that volatilization from the environmental water is low. Low bioaccumulation is suggested in aquatic organisms.

Many studies have been conducted to assess the toxic effects of *N*, *N*-dimethylformamide on organisms in the environment using indices including mortality, immobilization and growth inhibition. In a growth inhibition study of *N*, *N*-dimethylformamide in algae, the EC_{50} values ranged from 1,000 to 8,900 mg/L in freshwater algae. As the long-term toxicity of *N*, *N*-dimethylformamide to algae, the lowest value was 940 mg/L as the 96-hr NOEC for growth inhibition of a freshwater alga. The acute toxicity of *N*, *N*-dimethylformamide to invertebrates has been reported in freshwater water flea and midge and the 48-hr LC_{50} and EC_{50} (immobilization) values exceeded 1,000 mg/L. The long-term toxicity of *N*, *N*-dimethylformamide in the water flea has been reported and the 21-day NOEC for reproductivity ranged 1,000 to 1,500 mg/L. In fish, reliable acute toxicity data of *N*, *N*-dimethylformamide to freshwater fish are available in fathead minnow, Japanese killifish, bluegill, and rainbow trout, and the 96-day LC_{50} values of these species ranged from 7,100 to 10,600 mg/L, and the lowest value was 7,100 mg/L in bluegill. The long-term toxicity of *N*, *N*-dimethylformamide in Japanese killifish has been reported and the 21-day NOEC for growth was more than 102 mg/L. No reliable reports were obtained to evaluate effects of *N*, *N*-dimethylformamide on marine species.

The lowest value of toxicity in aquatic organisms is 940 mg/L as the 96-day NOEC for growth inhibition of the freshwater alga among the confirmed toxicity values.

In humans and experimental animals, *N*, *N*-dimethylformamide is rapidly absorbed via oral, inhalation and dermal routes. In human inhalation studies, the blood concentrations of unchanged *N*, *N*-dimethylformamide were rapidly decreased and not detected in 2 to 4 hours after exposure. *N*,

N-Dimethylformamide dermally applied in both liquid and vapor states showed high skin absorption in humans. *N*, *N*-Dimethylformamide was rapidly distributed to various organs such as liver, kidney, brain and adrenal grand as well as blood with almost homogeneous distributions after a single inhalation exposure to rats. In pregnant rats, orally administered *N*, *N*-dimethylformamide crossed the placenta and transferred to the fetus and amniotic fluid. In the metabolism of *N*, *N*-dimethylformamide, two *N*-methyl groups were sequentially hydroxylated and demethylated and excreted in the urine. *N*-Acetyl-*S*-(*N*-methylcarbamoyl) cysteine was also excreted.

In the acute toxicity of *N*, *N*-dimethylformamide to experimental animals, the oral LD_{50} values were 3,700 to 6,800 mg/kg in mice and 2,000 to 7,600 mg/kg in rats, and the LC_{50} values for inhalation exposure were 2,000 to 6,120 ppm (corresponding to 6,080 to 18,605 mg/m^3) in mice and 2,500 to 5,020 ppm (7,600 to 15,261 mg/m^3) in rats. The acute symptoms after *N*, *N*-dimethylformamide administration were body weight loss, restlessness, irritative symptoms and hepatic damages at high concentrations of exposure.

N, *N*-Dimethylformamide showed slight to moderate irritation to eyes in rabbits but not to the skin. No reports on skin sensitization were obtained in this investigation.

With regard to the repeated dose toxicity of *N*, *N*-dimethylformamide, it caused an increase in liver weight, hepatic degeneration and necrosis and blood biochemical changes in mice, rats, or rabbits in oral administration, inhalation exposure and dermal application. In addition, it resulted in renal disorder and changes in cardiac function and myocardium. The NOAEL for oral administration of 90 days to rats was 200 ppm (corresponding to 17.2 mg/kg/day) based on the results that hypercholesterolemia was observed at 1,000 ppm and above. The LOAEL for inhalation exposure of 18 months to mice was 25 ppm (corresponding to 76 mg/m^3) based on the results that hepatic hypertrophy, single cell necrosis, lipofuscin and hemosiderin pigment in Kupffer cells were found at 25 ppm (corresponding to 76 mg/m^3) and above.

With regard to the reproductive and developmental toxicity, 14-week oral administration of *N*, *N*-dimethylformamide to male and female mice (F_0) caused reduction in fertility and in F_2 mice decreases in the number of litters, body weight of surviving fetuses, malformation of the cranial and sternal bones. Oral administration and inhalation exposure to pregnant rats resulted in fetal body weight loss and skeletal variations in supraoccipital and sternebrae. Based on these results, *N*, *N*-dimethylformamide is considered to have reproductive and developmental toxicity as well as teratogenicity in mice and rats. The NOAEL of reproductive toxicity was 50 mg/kg/day for oral administration to rats, and 32 ppm (corresponding to 97 mg/m^3) for inhalation exposure to rats.

N, N-dimethylformamide was negative in most of the *in vitro* genotoxicity studies including reverse mutation assays in *Salmonella typhimurium*, chromosomal aberration tests in human and Chinese hamster cultured cells and in mouse lymphoma. Of *in vivo* studies, negative results were obtained in a micronucleus test in mice and sex-linked recessive lethal test in *Drosophila*. The overall evaluation of the available data indicates that *N, N*-dimethylformamide is not genotoxic.

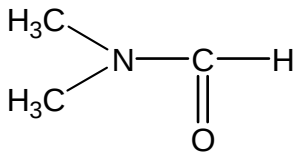
Regarding carcinogenicity of *N*, *N*-dimethylformamide, in a 2-year inhalation exposure studies in mice

and rats, hepatocellular adenoma and carcinoma and hepatic blastoma were increased in males and females mice at 200 ppm (corresponding to 152 mg/m^3) and above, and hepatocellular adenoma was observed in male and female rats at 400 ppm (corresponding to 1,216 mg/m^3) and above. *N*, *N*-dimethylformamide is considered carcinogenic in experimental animals with inhalation exposure. *N*, *N*-dimethylformamide has been categorized as Group 3 (not classifiable as to its carcinogenicity to humans) by the IARC.

Contents

1. Identity of the substance

1.1 Chemical name	:	*N*, *N*-Dimethylformamide
1.2 Class reference number in Chemical Substance Control Law[1)]	:	2-680
1.3 PRTR[2)] **Number (Law for PRTR and Promotion of Chemical Management)**	:	1-172
1.4 CAS registry number	:	68-12-2
1.5 Structural formula		



1.6 Molecular formula	:	C_3H_7NO
1.7 Molecular weight	:	73.09

2. General Information

2.1 Synonyms

Dimethyl formamide, Formyl dimethylamine, DMF

2.2 Purity

>99% (Commercial products) (CERI/Japan, 2002)

2.3 Impurities

Formic acid, Dimethylamine (Commercial products) (CERI/Japan, 2002)

2.4 Additives/Stabilizers

No additives and stabilizers (Commercial products) (CERI/Japan, 2002)

2.5 Current regulations in Japan[3)]

Law for PRTR and Promotion of Chemical Management	Class I-designated chemical substance

[1)] The Low Concerning the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc., Japan. Provisional translation is available on Internet at: http://www.safe.nite.go.jp/english/kasinn/kaiseikasinhou.html

[2)] Pollutant Release and Transfer Register

[3)] As this document covers basic information on Japanese regulations (unofficial translations), you should confirm the details using it.

Law Concerning The Examination And Regulation Of Manufacture, Etc. Of Chemical Substances (Chemical Substances Control Law):	Designated chemical substance (Type II monitoring chemical substance)
Waterworks Law:	Dangerous goods class IV second oil division
Industrial Safety and Health Law:	Dangerous substance inflammable substance, Harmful substance whose name is to be indicated, Hazardous substance to be notified in terms of whose name, Second-class organic solvent
Law Relating to the Prevention of Marine Pollution and Maritime Disasters:	Noxious liquid substance category D
Ship Safety Law:	Flammable liquid
Civil Aeronautics Law:	Flammable liquid
Port Regulation Law:	Flammable liquid

3. Physico-chemical properties

Appearance:	Colorless to slightly yellow liquid	(U.S.NLM:HSDB, 2002)
Melting point:	-61°C	(IPCS, 2000 ; Merck, 2001)
Boiling point:	153°C	(IPCS, 2000 ; Merck, 2001)
Flash point:	58°C (closed-cup) 67°C (open-cup)	(IPCS, 2000) (Merck, 2001)
Ignition point:	445°C	(IPCS, 2000)
Explosion limit:	2.2-15.2 vol% (100°C, in air)	(IPCS, 2000)
Specific gravity:	0.9445 (25°C /4°C)	(Merck, 2001)
Vapor density:	2.51 (air = 1)	
Vapor pressure:	380 Pa (20°C), 1,340 Pa (40°C)	(Verschueren, 2001)
Partition coefficient:	log Kow (*n*-octanol/water) = -1.01(measured), -0.93 (estimated)	(SRC:KowWin, 2002)
Dissociation constant:	pKa = -0.01 (20°C)	(U.S.NLM:HSDB, 2002)
Mass spectrum:	Main mass fragments m/z 73 (base peak = 1.0), 44 (0.86), 30 (0.22)	(NIST, 1998)
Soil adsorption coefficient:	Koc＝7 (estimated)	(U.S.NLM:HSDB, 2002)
Solubility:	water: freely soluble	(U.S.NLM:HSDB, 2002)
	alcohols: soluble	(U.S.NLM:HSDB, 2002)
	acetone: soluble	(U.S.NLM:HSDB, 2002)
	benzene: soluble	(U.S.NLM:HSDB, 2002)

Henry's constant: 7.49×10^{-3} Pa·m^3/mol (7.39×10^{-8} atm·m^3/mol) (SRC:HenryWin, 2002)
(25°C, measured)

Conversion factor: (Air, 20°C) 1 ppm = 3.04 mg/m^3,
1 mg/m^3 = 0.329 ppm

4. Sources of release to the environment

4.1 Production and import

The production and import volume of *N, N*-dimethylformamide in Japan was 63,043 tons in F.Y. 2000 and 57,724 tons in F.Y. 2001 (METI/Japan, 2002, 2003). The production and import volume means shipment volume not including production volume for self consumption.

4.2 Uses

The estimated use pattern of *N, N*-dimethylformamide is shown in Table 4-1 (The Chemical Daily, 2003). *N, N*-Dimethylformamide is used as solvent, catalyst and gas absorbent.

Table 4-1 Estimated use patterns

Uses	Details
Solvent	Artificial leather, urethane synthetic leather
	Spandex fiber
	Analytic chemistry (solvent, formylation reagent)
	Organic synthesis (synthesis of dye and intermediate, agrichemicals, pharmaceuticals)
	Various polymers (in particular acrylonitrile-type polymer)
	Specific ink, printing for textile (dissolution of dye, rhodamine and Victorian blue combined with phosphotungstic acid)
Catalyst	Acetylation of cellulose
Gas absorbent	Butadiene, acetylene, ethylene, propylene, sulfurous acid, Hydrogen sulfide, Hydrocyanic acid, Boron trifluoride, Sulfur trioxide, etc. Nitrogen, Hydrogen and saturated hydrocarbon are rarely absorbed.

(The Chemical Daily, 2003)

4.3 Releases

4.3.1 Releases under PRTR system

According to "Total Release and Transfers for the Fiscal Year 2001 (hereafter the 2001 PRTR Data)" under the PRTR system (METI/Japan and MOE/Japan, 2003a), 6,315 tons of *N, N*-dimethylformamide was released into air, 289 tons into public water, 8,971 tons was transferred as wastes and 954 tons was released into sewer from the business institutions required to report their releases and transfer for a year. No *N, N*-dimethylformamide was reported to be released into land. In addition, it is estimated that 19,344 tons of *N, N*-dimethylformamide was released from the business institutions in the business categories designated under the PRTR system but were exempted from notification, 140 tons from the business categories outside the scope of the PRTR system, and 138 kg from households. No estimation was made for the amounts of

releases from mobile sources.

a. Release and transfer from the business categories within the scope of PRTR system

The amounts of releases into the environmental media (air, water and land) and transfer by the designated industries estimated from the 2001 PRTR Data are shown in Table 4-2. As METI/Japan and MOE/Japan (2003a) do not provide the amounts of releases by environmental media from the business institutions exempted from notification, the releases from the business institutions exempted for notification were calculated based on the assumption that ratios of releases into the air, water and land were the same as those obtained by notification (NITE/Japan, 2004).

Table 4-2 Releases and transfer of *N, N*-dimethylformamide to environmental media by industries (tons/year)

Business Category	By Notification					Notification Exempted			Total amount of releases by notification and by estimation	
	Release			Transfer		Release (estimated)[1)]				
	Air	Water	Land	Sewer	Wastes	Air	Water	Land	Total release[3)]	Ratio (%)
Plastic products	2,845	85	0	327	996	12,785	586	0	16,300	62.8
Apparel and other textile goods	0	0	0	0	0	4,478	205	0	4,683	18.0
Textile mill products	1,133	44	0	511	1,492	429	20	0	1,626	6.3
Chemical and allied products	1,094	116	0	110	5,743	129	6	0	1,345	5.2
Electrical machinery, equipment and supplies	924	0	0	0	199	195	9	0	1,128	4.3
Rubber products	247	30	0	6	75	36	2	0	315	1.2
Other Industries	27	0	0	0	64	189	9	0	225	0.9
Leather tanning, leather and fur products	9	0	0	0	0	123	6	0	138	0.5
Others[2)]	36	14	0	0	401	130	6	0	587	2.3
Total[3)]	6,315	289	0	954	8,971	18,495	848	0	25,947	100

(NITE/Japan, 2004)

1) Based on the assumption that ratios of releases into the air, water and land were the same as those of the releases obtained by notification, the amounts of releases from the business institutions exempted from notification were estimated.

2) "Others" indicates the total release in the business categories other than the above.

3) The total may not correspond with the sum of rounded values in each column of the table.

The release and transfer of less than 0.5 tons was mentioned as "<0.5" without exception.

Based on the production volume and the emission factor in manufacturing sites of *N, N*-dimethyl

formamide in 2001 (Japan Chemical Industry Association, 2002), the amount of releases into the air is estimated to be 1 tons per year (NITE/Japan, 2004). Therefore, based on the 2001 PRTR Data, most of the releases of *N, N*-dimethylformamide from the business categories within the scope of PRTR system are considered to occur not during the manufacturing process but during the production process.

b. Releases from the non-designated business categories, households and mobile sources

Based on the 2001 PRTR Data, the amounts of release from the non-designated business categories and households are shown in Table 4-3. As METI/Japan and MOE/Japan (2003a) did not provide the amounts of releases by environmental media, those amounts were estimated considering its use and physicochemical properties (NITE/Japan, 2004)。

It was estimated that 140 tons of *N, N*-dimethylformamide was released as agricultural adjuvants into the air from the non-designated business categories and households, assuming that *N, N*-dimethylformamide was released into the air considering its physicochemical properties (METI/Japan and MOE/Japan, 2003b). The amounts of *N, N*-dimethylformamide releases from mobile sources are outside the scope of estimation required under PRTR (METI/Japan and MOE/Japan, 2003b).

Table 4-3 Releases of *N, N*-dimethylformamide from the non-designated industries and households (tons/year) into environmental medium

	Air	Water	Land
Non-designated business categories[1)]	140	0	0
Households[1)]	＜0.5	0	0
Total[2)]	140	0	0

(NITE/Japan, 2004)

1) The distribution to air, water and land was considered from the use and the physicochemical property.

2) The total may not correspond with the sum of rounded values in each column of the table.

"<0.5" indicates less than 0.5 tons

4.3.2 Releases from other sources

Other information on *N, N*-dimethylformamide release sources than estimations based on the 2001 PRTR Data was not obtained in this investigation. CICAD reported the possibility that *N, N*-dimethylformamide was formed by photolysis of dimethylamine and trimethylamine (IPCS, 2001), but the details were unknown and this reaction was not included in the routes of release.

4.4 Estimated routes of releases

Considering the information that *N, N*-dimethylformamide is used as solvent and catalyst, and based on the 2001 PRTR Data, the main release route is considered through emissions in the use process of *N, N*-dimethylformamide and products including *N, N*-dimethylformamide.

As the scenario of *N, N*-dimethylformamide releases in Japan, it was estimated that 24,951 tons of *N,*

N-dimethylformamide was released annually into the air, and 1,137 tons into water. Releases into the environment after processing of wastes at waste disposal facilities were not considered for estimation of the amount transferred as wastes and that transferred into sewers.

5. Environmental fate

5.1 Stability in the atmosphere

a. Reaction with OH radical

The reaction rate constant of *N, N*-dimethylformamide with OH radical is 1.75×10^{-11} cm^3/molecule-sec (25°C, estimated value) in the tropospheric air (SRC: AopWin, 2003). On the assumption of OH radical concentration of 5×10^5 to 1×10^6 molecule /cm^3, the half-life is calculated as 0.5 to 1 day.

b. Reaction with ozone

No reports on reaction of *N, N*-dimethylformamide with ozone were obtained in this investigation.

c. Reaction with nitrate radical

No reports on reaction of *N, N*-dimethylformamide with nitrate radical were obtained in this investigation.

d. Direct degradation by sunlight

As *N, N*-dimethylformamide does not absorb light at 290 nm and above, *N, N*-dimethylformamide is not degraded directly by sunlight in the air (U.S. NLM:HSDB, 2001).

5.2 Stability in water

5.2.1 Abiotic degradation

As *N, N*-dimethylformamide has an amide group, it is probably hydrolyzed in the aquatic environment, though the rate is extremely slow (U.S. NLM: HSDB, 2001). The half-life of *N, N*-dimethylformamide by hydrolysis was estimated to be one year and more (SRC:HydroWin, 2003).

5.2.2 Biodegradation

N, N-dimethylformamide is ranked as a "not readily biodegradable substance" based on the result of the aerobic biodegradation study required under the Chemical Substance Control Law. The study result indicated that the degradation rate of *N, N*-dimethylformamide was 4% in biological oxygen demand (BOD) determination under the conditions of 100 mg/L of test substance concentration, 30 mg/L of activated sludge concentration and 2 weeks of test period. The degradation rates were 9% and 4% in the total organic carbon (TOC) determination and by measurement with gas chromatography (GC), respectively (MITI/Japan, 1975).

However, it was reported that *N, N*-dimethylformamide was biodegraded in aerobic condition as described below.

(1) In an acclimated and unacclimated river die away test, *N,N*-dimethylformamide at an initial concentration of 30 mg/L was completely eliminated in 3 and 6 days, respectively (U.S. NLM: HSDB, 2001).

(2) At 400 mg/L, 95% and above of organic carbon in *N, N*-dimethylformamide was degraded in 7 days (Verschueren, 2001).

(3) Although the detailed experimental conditions were unknown, 100% of organic carbon in *N, N*-dimethylformamide at 20 mg/L was degraded within 21 days after a 14-day induction period in an urban wastewater (Verschueren, 2001).

No reports were obtained on anaerobic biodegradation of *N, N*-dimethylformamide in this investigation.

Based on the information described above, it is considered that *N, N*-dimethylformamide is biodegraded in specific aerobic conditions associated with acclimation.

5.2.3 Removal in sewage treatment

It was reported that *N, N*-dimethylformamide of 99% and above was easily biodegraded in an acclimated sewage facility (Howard, 1993).

5.3 Behavior in the aquatic environment

N, N-Dimethylformamide is miscible with water and Henry's constant (7.49×10^{-3} Pa·m^3/mol, 25°C) is low.

Based on the information described here and in Section 5.2.2, it is assumed that *N, N*-dimethylformamide released into the aquatic environment is eliminated by biodegradation. It is considered that volatilization from the aquatic environment is low.

5.4 Bioaccumulation

N, N-Dimethylformamide is ranked as " non- or low bioaccumulative substance " based on the result of an 8-week bioaccumulation study in carp required under the Chemical Substance Control Law. The study result indicated that the bioaccumulation factors of *N, N*-dimethylformamide were 0.3 to 0.8 and 0.3 to 1.2 at 20 and 2 mg/L of *N, N*-dimethylformamide concentration in water, respectively (MITI/Japan, 1975).

6. Effects on organisms in the environment

6.1 Effects on aquatic organisms

6.1.1 Microorganisms

The toxicity studies of *N, N*-dimethylformamide for microorganisms are summerized in Table 6-1.

Regarding the toxicity of *N, N*-dimethylformamide for microorganisms, it has been reported that *N,N*-dimethylformamide inhibited luminescence of marine luminescent bacteria *Photobacterium phosphoreum*, and that the 5-min EC_{50} value was 20,000 mg/L (Curtis et al., 1982).

Table 6-1 Toxicity of *N, N*-dimethylformamide for to microorganisms

Species	Tem-perature (°C)	Endpoint		Concentration (mg/L)	Reference
Bacteria *Photobacterium phosphoreum* (marine luminescent bacterium)	15	5-min EC_{50}	luminescence inhibition	20,000	Curtis et al., 1982

6.1.2 Algae

The toxicity studies of *N, N*-dimethylformamide for algae are summerized in Table 6-2.

Growth inhibition studies of *N, N*-dimethylformamide in freshwater algae *Selenastrum capricornutum* and *Chlorella* were reported, and all the EC_{50} values exceeded 1,000 mg/L.

As the long-term toxicity of *N, N*-dimethylformamide, the NOEC values (72 to 96 hours) for growth inhibition in *Selenastrum capricornutum* were more than 1,000 mg/L (EA/Japan, 1996) and 940 mg/L (El Jay, 1996), and in *Chlorella* was 4,700 mg/L (El Jay, 1996).

In addition, the 10 to 14-day EC_{50} values for growth inhibition have been reported on five blue green algae, and the EC_{50} values for *Anabaena* sp., *A. cylindrical*, *A. variabilis* and *Nostoc* sp. were below 470 mg/L and for *A. inaequalis* was 5,700 mg/L (Stratton, 1987).

No reports in marine algae were obtained.

Table 6-2 Toxicity of *N, N*-dimethylformamide for algae

Species	Method/ Condition	Tem-perature (°C)	Endpoint		Concen-tration (mg/L)	Reference
Freshwater species						
Selenastrum capricornutum[1)] (green alga)	OECD 201 GLP Static	22.8-23.2	72-hr EC_{50} 24 to 48-hr EC_{50} 24 to 72-hr EC_{50} 72-hr NOEC 24 to 48-hr NOEC 24 to 72-hr NOEC	Growth inhibition biomass growth rate growth rate biomass growth rate growth rate	>1,000 >1,000 >1,000 ≧1,000 ≧1,000 ≧1,000 (a, n)	EA/Japan, 1996
	Static	21±1	96-hr NOEC	Growth inhibition chlorophyll a	940 (n)	El Jay, 1996
Chlorella pyrenoidosa (green alga)	Static	25±1	10 to 14-day EC_{50}	Growth inhibition	8,900 (n)	Stratton & Smith, 1988
Chlorella vulgalis (green alga)	Static	21±1	96-hr NOEC	Growth inhibition chlorophyll a	4,700 (n)	El Jay, 1996
Anabaena sp. (blue green alga)	Static	25±1	10 to 14-day EC_{50}	Growth inhibition	< 470	Stratton, 1987

Species	Method/ Condition	Tem-perature (°C)	Endpoint		Concen-tration (mg/L)	Reference
Anabaena cylindrical (blue green alga)	Static	25±1	10 to 14-day EC_{50}	Growth inhibition	< 470	Stratton, 1987
Anabaena variabilis (blue green alga)	Static	25±1	10 to 14-day EC_{50}	Growth inhibition	< 470	Stratton, 1987
Anabaena inaequalis (blue green alga)	Static	25±1	10 to 14-day EC_{50}	Growth inhibition	5,700	Stratton, 1987
Nostoc sp. (blue green alga)	Static	25±1	10 to 14-day EC_{50}	Growth inhibition	< 470	Stratton, 1987

(a, n): The measured concentration of test substance was within ±20% of the nominal concentration, then, the nominal one is shown instead the measured one, (n): Nominal concentration

1) Current scientific name: *Pseudokirchneriella subcapitata*

6.1.3 Invertebrates

The toxicity studies of *N, N*-dimethylformamide for invertebrates are summerized in Table 6-3.

The acute toxicity of *N, N*-dimethylformamide has been reported in crustacean water flea, and the 24 and 48-hr LC_{50} values ranged between 12,000 and 16,000 mg/L and the 24 and 48-hr EC_{50} (immobilization) ranged from 1,000 to 26,300 mg/L, respectively. The 48-hr LC_{50} in insect bloodworm was 33,500 mg/L, and the 48-hr EC_{50} (behavior, etc.) was 36,200 mg/L (Poirier et al., 1986; Ziegenfuss et al., 1986).

The long-term toxicity of *N, N*-dimethylformamide to reproductivity in *Daphnia magna* has been reported, and the 21 and 28-day NOECs were more than 1,000 mg/L (EA/Japan, 1996) and 1,100 mg/L (LeBlanc and Surprenant, 1983), respectively.

No reports on toxicity of *N, N*-dimethylformamide to marine species were obtained in this investigation.

Table 6-3 Toxicity of *N, N*-dimethylformamide for invertebrates

Species	Size/ Growth stage	Method/ Condition	Tem-perature (°C)	Hardness (mg $CaCO_3$ /L)	pH	Endpoint	Concen-tration (mg/L)	Reference
Freshwater species								
Daphnia magna (crustacea, water flea)	<24 hours	Static	21±1	165±15	7.9-8.3	24-hr LC_{50} 48-hr LC_{50}	16,000 12,000 (n)	LeBlanc & Surprenant, 1983
		Flow-through	21±1	165±15	7.9-8.3	28-day NOEC 28-day LOEC Reproduction	1,100 2,400 (n)	
		ASTM[1)] Static	20-23	120-250	7.0-8.5	24-hr EC_{50} 48-hr EC_{50} Immobilization	19,800 15,700 (m)	Adams & Heidolph, 1985
		ASTM[1)] Semi-static	21-23	240-310	7.2-8.5	21-day NOEC 21-day LOEC Reproduction	1,500 3,000 (m)	

Species	Size/ Growth stage	Method/ Condition	Tem-perature (°C)	Hardness (mg $CaCO_3$ /L)	pH	Endpoint	Concen-tration (mg/L)	Reference
		Static	20.9-23.4	238-280	6.1-8.3	48-hr LC_{50}	14,400 (n)	Ziegenfuss et al., 1986
		Static	20.5	40.4-56.3	7.04 - 7.97	24-hr EC_{50} 48-hr EC_{50} Immobilization	26,300 14,500 (a, n)	Poirier et al., 1986
		OECD 202 GLP Semi-static	19.9-20.2	50	8.0-8.2	24-hr EC_{50} 48-hr EC_{50} Immobilization	> 1,000 > 1,000 (a, n)	EA /Japan, 1996
		OECD 202 GLP Semi-static	19.8-20.7	50	7.8-8.3	21-day NOEC 21-day LOEC Reproduction	≧1,000 > 1,000 (a, n)	
Chironomus tentas (insect, one of midge)	10-14 days 2nd instar larva	Static	20.9-23.4	238-280	6.1-8.3	48-hr LC_{50}	33,500 (n)	Ziegenfuss et al., 1986
Paratanytarsus parthenogeneticus (insect, one of midge)	(within 24 hours) 3rd instar larva	Static	23	40.4-56.3	7.04 - 7.97	24-hr EC_{50} 48-hr EC_{50} Behavior, etc.	46,800 36,200 (a, n)	Poirier et al., 1986

(a, n): As the measured concentrations of test substance was within ±20% of the nominal concentrations, the nominal concentrations are shown in this table, (m): Measured concentration, (n): Nominal concentration

1) Test guideline by the American Society for Testing and Materials

6.1.4 Fish

The toxicity studies of *N, N*-dimethylformamide for fish are summerized in Table 6-4.

Reliable acute toxicity data of *N, N*-dimethylformamide to freshwater fish are available in fathead minnow, Japanese killifish, bluegill and rainbow trout. The 96-day LC_{50} values of these species ranged from 100 to 10,600 mg/L and the lowest value was 7,100 mg/L in bluegill, which was measured in a flow-through condition according to the test method of U.S. EPA (Poirier et al., 1986).

The long-term toxicity of *N, N*-dimethylformamide in Japanese killifish has been reported, and the 21-day NOEC for growth was more than 102 mg/L (EA/Japan, 1996).

A two-generation study was conducted in fathead minnow and brook trout, and the maximum acceptable hazardous concentrations (MATC) were 5 to 10 and 43 to 98 mg/L, respectively (Cardwell et al., 1978). However, bacteria were observed in the study, which might have an effect on the study results. Therefore, these values are not included in this assessment.

No reports on toxicity of *N, N*-dimethylformamide to marine fish were obtained in this investigation.

Table 6-4 Toxicity of *N, N*-dimethylformamide for fish

Species	Growth stage	Method/ Condition	Temperature (°C)	Hardness (mg $CaCO_3$/ L)	pH	Endpoint	Concentration (mg/L)	Reference
Freshwater species								
Pimephales promelas (fathead minnow)	0.047± 0.022 g	Flow-through	23.3 ±1.7	40.4-56.3	7.04-7.97	96-hr LC_{50} 96-hr EC_{50} Behavior, etc.	10,600 10,600 (a, n)	Poirier et al., 1986
Oryzias latipes (Japanese killifish)	2.1 cm 0.18 g	OECD 203 Semi-static	23.3-24.2	50	7.4-8.0	96-hr LC_{50}	> 100 (a, n)	EA/Japan, 1996
	2.2 cm 0.16 g	OECD 204 GLP Flow-through	23.7-24.1	50	7.4-7.9	21-day NOEC Growth	≧102 (a, n)	
Lepomis macrochirus (bluegill)	0.912± 0.350 g	Flow-through	19.8 ±2.3	40.4-56.3	7.04-7.97	96-hr LC_{50} 96-hr EC_{50} Behavior, etc.	7,100 7,100 (a, n)	Poirier et al., 1986
Oncorhynchus mykiss (rainbow trout)	5.08± 1.97 g	Flow-through	12.7 ±1.0	40.4-56.3	7.04-7.97	96-hr LC_{50} 96-hr EC_{50} Behavior, etc.	9,800 9,800 (a, n)	Poirier et al., 1986

(a, n): As the measured concentrations of test substance was within ±20% of the nominal concentrations, the nominal concentrations are shown in this table, (n): Nominal concentration

6.1.5 Other aquatic organisms

No reports on the toxicity of *N, N*-dimethylformamide in other aquatic organisms (e.g., amphibians) were obtained in this investigation.

6.2 Effects on terrestrial organisms

6.2.1 Microorganisms

The toxicity studies of *N, N*-dimethylformamide for microorganisms are summerized in Table 6-5.

The toxicity of *N, N*-dimethylformamide to three kinds of bacteria was studied on colony growth, and the EC_{50} values ranged from 4,800 to 10,200 mg/L (Stratton, 1985).

Table 6-5 Toxicity of *N, N*-dimethylformamide for microorganisms

Species	Temperature (°C)	Endpoint		Concentration (mg/L)	Reference
Pythium ultimum (phytopathogenic fungi)	25	EC_{50}	Growth inhibition	10,200	Stratton, 1985
Sclerotinia homeocarpa (phytopathogenic fungi)	25	EC_{50}	Growth inhibition	4,800	Stratton, 1985
Pestalotia sp. (phytopathogenic fungi)	25	EC_{50}	Growth inhibition	6,000	Stratton, 1985

6.2.2 Plants

No reports on the toxicity of *N, N*-dimethylformamide in plants were obtained in this investigation.

6.2.3 Animals

No reports on the toxicity of *N, N*-dimethylformamide in animals were obtained in this investigation.

6.3 Summary of effects on organisms in the environment

Many studies have been conducted to assess the hazardous effects of *N, N*-dimethylformamide on environmental organisms using indices including mortality, immobilization and growth inhibition.

In microorganisms, *N,N*-dimethylformamide inhibited luminescence of marine luminescent bacteria *Photobacterium phosphoreum*, and that the 5-min EC_{50} value was 20,000 mg/L.

In a growth inhibition study of *N, N*-dimethylformamide in algae, the EC_{50} ranged from 1,000 to 8,900 mg/L in *Selenastrum capricornutum* and *Chlorella*. In addition, the 10 to 14-day EC_{50} values for growth inhibition for *Anabaena* sp., *A. cylindrical*, *A. variabilis* and *Nostoc* sp. were below 470 mg/L and for *A. inaequalis* was 5,700 mg/L. As the long-term toxicity of *N, N*-dimethylformamide to algae, the confirmed lowest value was 940 mg/L as the 96-hr NOEC for growth inhibition in *Selenastrum capricornutum*.

The acute toxicity of *N, N*-dimethylformamide to invertebrates has been reported in water flea and bloodworm and the values exceeded 1,000 mg/L. The long-term toxicity of *N, N*-dimethylformamide in *Daphnia magna* has been reported and the NOEC for reproductivity ranged 1,000 to 1,500 mg/L.

In fish, reliable acute toxicity data of *N, N*-dimethylformamide to freshwater fish are available in fathead minnow, Japanese killifish, bluegill, and rainbow trout and the 96-day LC_{50} of these species ranged from 7,100 to 10,600 mg/L and the lowest value was 7,100 mg/L in bluegill. The long-term toxicity of *N, N*- dimethylformamide in Japanese killifish has been reported and the 21-day NOEC for growth was more than 102 mg/L. No reliable reports were obtained to evaluate effects of *N, N*-dimethylformamide on marine species.

In terrestrial organisms, toxicity of *N, N*-dimethylformamide to three kinds of bacteria was studied on colony growth and the toxicity ranged from 4,800 to 10,200 mg/L.

The lowest value of toxicity in aquatic organisms is 940 mg/L as the 96-day NOEC for growth inhibition in alga, *Selenastrum capricornutum.*

Although formal classification criteria is not used in this investigation, it can be considered that the acute toxicity values of *N, N*-dimethylformamide to aquatic organisms does not corresponding to the GHS acute toxicity hazard category.

7. Effects on human health

7.1 Kinetics and metabolism

Studies on the kinetics and metabolism of *N, N*-dimethylformamide in experimental animals are summarized in Table 7-1.

a. Absorption

In the studies of *N, N*-dimethylformamide in humans and experimental animals, a rapid increase and

subsequent decrease of *N, N*-dimethylformamide concentrations in the blood were observed.

Absorption of orally administered *N, N*-dimethylformamide was reported in only one study. In an oral administration study of ^{14}C-labeled *N, N*-dimethylformamide to pregnant rats at a single dose of 100 mg/kg on gestation day 12, 14% of the given dose was detected in the gastrointestinal tract in 0.5 hours after administration (Saillenfait et al., 1997).

Many inhalation exposure studies were conducted in humans. Following inhalation exposure studies at concentrations of 53 and 82 ppm (corresponding to 161 and 249 mg/m^3) for 2 hours (Eben and Kimmerle, 1976), inhalation exposure at concentrations of 26 and 87 ppm (corresponding to 79 and 264 mg/m^3) for 4-hour and exposure at a concentration of 21 ppm (corresponding to 64 mg/m^3) for 5-day (4 hours/day) (Kimmerle and Eben, 1975b) were reported. In all these studies, the blood concentration of unchanged *N, N*-dimethylformamide was rapidly decreased and was not detected in 2 to 4 hours after exposure.

N, N-dimethylformamide was exposed to rats at concentrations of 87 and 209 ppm (corresponding to 264 and 635 mg/m^3) for 2 hours. The blood concentration of *N, N*-dimethylformamide reached the peak immediately after exposure, and then decreased rapidly. The concentrations in females were higher than those in males (Eben and Kimmerle, 1976). In an exposure study to rats at a concentration of 200 ppm (608 mg/m^3) for 5 days (2 hours/day), the blood concentration was not increased and showed similar values from exposure day 1 to exposure day 5 (Eben and Kimmerle, 1976). In contrast, in another exposure study in rats at a concentration of 2,005 ppm (6,095 mg/m^3) for 3 hours, the blood concentrations kept constant to 4.5 hours after exposure, and then decreased. However, *N, N*-dimethylformamide was detected at 3 days after exposure, which suggested the possibility of saturation (Kimmerle and Eben, 1975a).

In an inhalation exposure study of *N, N*-dimethylformamide in dogs at concentrations of 210 to 240 ppm (corresponding to 638 to 730 mg/m^3) for 2 hours, the blood concentration of *N, N*-dimethylformamide reached the peak immediately after the completion of exposure, and then decreased rapidly (Eben and Kimmerle, 1976). However, in another inhalation exposure study at a concentration of 21 ppm (64 mg/m^3) for 4 weeks (6 hours/day, 5 days/week), *N, N*-dimethylformamide was detected from the blood at 2 weeks after the initiation of exposure (Kimmerle and Eben, 1975a). In the inhalation exposure study using dogs at a concentration of 59 ppm (corresponding to 179 mg/m^3) in males and 23 ppm (70 mg/m^3) in females for 5 days (6 hours/day), the blood concentration was increased until exposure day 4 (Kimmerle and Eben, 1975a).

N, N-Dimethylformamide in liquid and vapor showed high skin absorption in humans. In a 4-hour exposure study in 13 healthy male volunteers at concentrations of 6.2 to 7.1 ppm, the ratios of skin absorption to total absorption through the skin and lung was approximately 40% in average (Nomiyama et al., 2001). In 3 workers who were engaged in a routine work for 8 hours and exposed to *N, N*-dimethylformamide at concentrations from 12.1 to 40.0 mg/m^3, the percentages of skin absorption were 62%, 26% and 27%, respectively. It was reported that as the respiratory volume was larger in 8-h exposure under working conditions, the percentage of skin absorption was low (Miyauchi et al., 2001).

b. Distribution

N, N-Dimethylformamide was rapidly distributed to various organs. In pregnant rats, *N, N*-

dimethylformamide crossed the placenta and transferred to the fetus and amniotic fluid.

In an oral administration study of ^{14}C-*N, N*-dimethylformamide in pregnant rats at a single dose of 100 mg/kg on gestation day 12, *N, N*-dimethylformamide reached the maximum concentration within one hour and distributed to various tissues. The concentration of radioactivity in tissues were fetus > amniotic fluid > maternal liver > placenta in the order, ranging from 6.52% to 2.41% (Saillenfait et al., 1997). In a study using lactating rats, the radioactivity in milk were similar to the plasma concentration through 24 hours, i.e., the concentration reached the peak in 2 hours after administration and reduced to approximately 6% of the peak concentration by 24 hours after administration. The plasma concentrations in lactating rats were slightly lower than those in pregnant rats and it took longer time to reach the peak. The concentration in the gastrointestinal tract of lactating rats at 1 hour after administration was approximately double of that of pregnant rats (Saillenfait et al., 1997).

In a single inhalation exposure to rats at concentrations of 565 and 2,250 ppm (corresponding to 1,718 and 6,840 mg/m^3), the concentrations immediately after exposure in the blood, liver, kidney, brain and adrenal grand showed almost homogeneous distributions (Lundberg et al., 1983).

In a single intraperitoneal injection to rats at a dose of 1 mL/kg, approximately 4% of the given dose was detected in the blood and approximately 7% in other organs at 24 hours after administration (Scailteur et al., 1984).

c. Metabolism/Excretion

Many metabolism studies of *N, N*-dimethylformamide have been reported. In the metabolism of *N, N*-dimethylformamide, two *N*-methyl groups were sequentially hydrated and demethylated to form *N*-hydroxymethyl-*N*-methylformamide (DMF-OH), *N*-methylformamide (NMF), *N*-hydroxymethylformamide (NMF-OH) and formamide (FA) detected in the urine. *N*-acetyl-S-(*N*-methylcarbamoyl) cysteine (AMCC) was also detected (Mraz et al., 1989; Scailteur et al., 1984). In a comparison study of urinary metabolites between rodents and humans, the percentages of AMCC in humans was higher than in rodents. The excretion rate varied in animal species, and AMCC were almost completely excreted within 24 hours in mice, but it took longer time to excrete AMCC compared with NMF in rats, hamsters and humans (Mraz et al., 1989).

In oral administration to *N, N*-dimethylformamide to pregnant rats at a single dose on gestation day 12, concentrations of the unchanged *N, N*-dimethylformamide were 61% to 77% at 4 hours after administration and were reduced to 30% to 33% at 8 hours after administration. The concentrations of metabolites DMF-OH and NMF were 10% to 14% and 3% to 4% of the total radioactivity administered at 1 hour after administration, and increased to 40% to 47% and 9% to 13% at 8 hours after administration, respectively. Almost no AMCC and FA were detected (Saillenfait et al., 1997). The concentration of radioactivity in the placenta and fetus of pregnant rats were 64% to 70% and 79% to 93% of the maternal plasma concentrations until 8 hours after administration, respectively. However, at 48 hours after administration, these concentrations were approximately 3 to 4-fold of maternal plasma concentrations, suggesting that the excretion in the placenta and fetus was slow. The concentration in amniotic fluid was almost similar to maternal plasma concentration until 24 hours after administration, and decreased thereafter (Saillenfait et

al., 1997).

In addition to the metabolites described above, 3-methyl-5-isopropylhydantoin (MIH) was detected as a metabolite in humans (Angerer et al., 1998).

Table7-1 Kinetic and metabolism of *N, N*-dimethylformamide

Species	Route	Dose	Results	Reference
Pregnant rat SD	Single oral administration Gestation day 12	[^{14}C]-labeled: 100 mg/kg	Absorption: 0.5 hours after administration: 14% of given dose was detected in the gastrointestinal tract. within 48 hours after administration: 3.4% excreted in feces Distribution: 1 hour after administration: distributed in all tissues (peak) Tissues with high concentration: maternal plasma and amniotic fluid Detected in other tissues (placenta, maternal kidney, liver, ovary and uterus) Metabolism: Parent substance (unchanged): 0-4 hours after administration: 61-77% of radioactivity administered 8 hours after administration: 30-33% of radioactivity administered Metabolites (DMF-OH and NMF): 1 hour after administration: 10-14% and 3-4% of total radioactivity 8 hours after administration: increased to 40-47% and 9-13% AMCC and FA: almost not detected Excretion: Radioactivity in all tissues: maintained high until 4 hours after administration and decreased gradually 8-24 hours: rapidly decreased Radioactivity in placenta and fetus: 0.5-8 hours: 64-70% and 79-93% of maternal concentration 48 hours after administration: approximately 3 to 4-fold of the plasma concentration, suggesting that excretion in the placenta and fetus was slow Radioactivity in amniotic fluid: Up to 24 hours: almost similar to the maternal plasma concentration 30 and 48 hours: lower than the maternal plasma concentration	Saillenfait et al., 1997

Species	Route	Dose	Results	Reference
Pregnant rat SD	Single oral administration Gestation day 18	[^{14}C]-labeled: 100 mg/kg	Absorption: Unchanged *N, N*-dimethylformamide: reached the peak 4-8 hours after administration, thereafter decreased DMF-OH and NMF: increased as time Distribution: Tissue concentration: fetus > amniotic fluid > maternal liver > placenta (6.52-2.41% of given dose) Metabolism: Unchanged *N, N*-dimethylformamide: 8 hours after administration: 73-93% of total radioactivity, 16 hours after administration: decreased to 14-21% Metabolite DMF-OH: 8 hours after administration: 1-11% of total radioactivity, 16 hours after administration: 41-55% Metabolites: AMCC and FA: plasma and tissue: less than 4% of total radioactivity Excretion: time course and tissue concentrations: similar to those after administration on gestation day 12. Radioactivity in the amniotic fluid: almost the same as the maternal plasma concentration	
Rat SD Female	Lactating period		Distribution: : Radioactivity in milk: the concentration reached the peak at 2 hours after administration and reduced to approximately 6% of the peak concentration after 24 hours Plasma concentration: slightly lower than the concentration of pregnant rat, delayed peak time Radioactivity in gastrointestinal tract: 1 hour after administration: approximately 2-fold of the concentration of pregnant rats Metabolism: Concentrations of unchanged *N, N*-dimethyl-formamide and its metabolites in milk after 1, 2 and 4 hours after administration were almost similar to maternal plasma concentrations Unchanged *N, N*-dimethylformamide: approximately 82-86% of total radioactivity DMF-OH and NMF: approximately 4-9% and 2% of total radioactivity AMCC and FA: around the detection limit	
Human	Inhalation Single dose 2 hours	53, 82 ppm (161, 249 mg//m^3)	Absorption: reached the peak immediately after exposure and decreased rapidly Metabolism: NMF concentration reached the peak at 4-5 hours after exposure. Excretion: *N, N*-dimethylformamide and NMF were detected at 4 hours after exposure but FA was not detected.	Eben & Kimmerle, 1976

Species	Route	Dose	Results	Reference
Human (20-50 years old)	Inhalation Single dose 4 hours	26, 87 ppm (79, 264 mg/m^3)	Absorption: 26, 87 ppm: blood unchanged *N, N*-dimethyl-formamide was rapidly reduced after exposure and was not detected at 2-3 hours after. Excretion: 26 ppm: not detected 87 ppm: excreted in 24 hours after the initiation of exposure, mean: 2.42 mg Metabolite NMF 26 ppm: the concentration was maintained constant until several hours after exposure 87 ppm: increased up to 3 hours after exposure NMF and FA: increased dose-dependently, a delayed tendency in excretion at high concentrations	Kimmerle & Eben, 1975b
Human (20-50 years old)	Inhalation Repeated dose: 5 days 4 hours/day	21 ppm (64 mg/m^3)	Absorption: unchanged *N, N*-dimethylformamide in blood concentration: rapidly decreased after exposure and not detected 4 hours after Excretion: not detected in the urine throughout the study period. NMF excretion varied between individuals, the concentration was maintained constant for several hours after exposure and decreased, 48 hours after not detected Throughout the study period, NMF and FA showed a similar pattern without accumulation	
Human Male: 6 smoker 4 nonsmoker	Inhalation	2.2-53.7 ppm (7-163 mg/m^3)	Metabolism: confirmed formation of 3-methyl-5-isopropylhydantoin (MIH) via *N*-methylcarbamoylized adduct in *N*-valine of hemoglobin MIH was detected from a globulin sample of an exposed worker.	Angerer et al., 1998
Rat Wistar Male and female	Inhalation 2 hours	87, 209 ppm (264, 635 mg/m^3)	Absorption: reached the peak immediately after exposure and decreased rapidly (in both males and females) The concentrations in females were higher than those in males. Metabolism: NMF: detected in the blood immediately after the initiation of exposure Excretion: *N, N*-dimethylformamide was not detected.	Eben & Kimmerle, 1976
	Inhalation 5 days 2 hours/day	200 ppm (608 mg/m^3)	Absorption: throughout the study period, the blood concentration of *N, N*-dimethylformamide was not increased. The changes of blood concentrations on exposure day 1 showed a similar tendency to that on exposure day 5. Metabolism: the NMF blood concentration change on exposure day 1 showed a similar tendency to that on exposure day 5 and no accumulation was found. Excretion: detected in 24 hours after the initiation of exposure throughout the study period. NMF concentrations in females were higher than those in males. FA concentrations were almost same.	

Species	Route	Dose	Results	Reference
Rat SD Female	Inhalation Single dose	565 ppm (690 mg/m^3) and 2,250 ppm (6,700 mg/m^3)	Distribution: Mean concentration in tissues (immediately after exposure): 565 ppm: Blood: 5.1, liver: 2.8, kidney: 3.1, brain: 3.1, adrenal gland: 2.1 (unit: μmol/g) Distributed evenly in the liver, kidney, brain and adrenal gland. 2,250 ppm: Blood: 13.2, liver: 9.8, kidney: 11.0, brain: 11.4, adrenal gland: 8.6 (unit: μmol/g) Metabolism: Metabolite NMF: 565 ppm: Gradually increased from immediately after exposure, reached the peak at 6 hours after exposure and below the detection limit at 20 hours after exposure. 2,250 ppm: Reached the peak at 20 hours after exposure Below the detection limit at 48 hours after exposure From immediately after exposure to 3 hours after, NMF formation was delayed at a concentration of 2,250 ppm compared with at a concentration of 565 ppm. At the point of the peak, concentrations were almost the same in tissues Excretion: 565 ppm: Immediately after exposure reached the peak, thereafter, gradually decreased and below the detection limit at 20 hours after exposure 2,250 ppm: From immediately after exposure to 6 hours after: Concentrations were same in all tissues, thereafter, decreased and below the detection limit at 48 hours after exposure Other: At a high concentration, metabolism to NMF was delayed, which is assumed that exposure at a high concentration inhibits demethylation	Lundberg et.al., 1983

Species	Route	Dose	Results	Reference
Rat Wistar	Inhalation Single dose 3 hours	21, 146, 2,005 ppm (4, 444, 6,095 mg/m^3)	Distribution: Blood concentration: 21 ppm: not detected immediately after exposure It was assumed that at a high concentration, metabolism saturation led a prolonged detection until 3 days after exposure 146 ppm: rapidly decreased after exposure and not detected at 21 hours after 2,005 ppm: Maintained almost at the same level until 4.5 hours, reduced thereafter, and detected for 3 days after exposure Metabolism: NMF: 146 and 2,005 ppm: increased after exposure, an increasing tendency was prolonged at a higher concentration Excretion: Parent compound: rapidly decreased at 24 hours after administration and not detected at 96 hours after administration. NMF and FA: increased until 48 hours after, and decreased thereafter. Increases in NMF and FA with decrease of parent compound	Kimmerle & Eben, 1975a
	Inhalation Single dose 6 hours	29, 170 ppm (88, 517 mg/m^3)	Distribution: The blood concentration showed a similar tendency to that in 3-h exposure Excretion: 29 and 170 ppm: not detected at 24 hours after exposure, NMF and FA were not detected at 48 and 72 hours after exposure, respectively (FA excretion was delayed from NMF excretion)	
Rat Wistar	Inhalation Repeated dose: 5 days 6 hours/day	350 ppm (1,064 mg/m^3)	Metabolism: Throughout the study period, the blood concentrations of *N, N*-dimethylformamide were constant without accumulation. *N, N*-dimethylformamide and NMF were not detected at 24 hours after exposure Excretion: Detected after 2nd exposure, and not detected after the completion of exposure. NMF and FA reached maximum after 2nd exposure, and after the completion of exposure. NMF and FA were detected until 24 and 48 hours after (FA excretion was delayed from NMF excretion) No accumulation	
Dog beagle Male and female	Inhalation Repeated dose: 5 days 6 hours/day	Male: 59 ppm (corresponding to 179 mg/m^3) Female: 23 ppm (corresponding to 70 mg/m^3)	4 days after the initiation of exposure: increase in the blood concentration	
Dog beagle	Inhalation Single dose 2 hours	210-240 ppm (638-730 mg/m^3)	Absorption: reached the peak immediately after exposure and decreased rapidly. NMF concentration reached the peak at 4 hours after exposure. Excretion: *N, N*-dimethylformamide, NMF and FA were detected in the urine for 24 hours immediately after exposure.	Eben & Kimmerle, 1976

Species	Route	Dose	Results	Reference
Dog beagle Male and female	Inhalation Repeated dose: 5 days 6 hours/day	23, 350 ppm (70, 1,064 mg/m^3)	Absorption: the blood concentration of NMF was increased from the initiation of exposure to exposure day 4, and detected for 4 days after the completion of exposure. Excretion: minimal parent compound was detected in the urine and NMF and FA were increased after the initiation of exposure and FA was detected for a longer period than NMF. NMF and FA showed a similar tendency to those at 350 ppm.	
	Inhalation Repeated dose: 4 weeks 6 hours/day 5 days	21 ppm (64 mg/m^3)	Absorption: Detected in the blood in males and females 2 weeks after exposure and later. Metabolism: NMF was increased throughout the study period, and significantly increased in males, slightly higher in males. Excretion: With exposure, urinary NMF was also was higher in males than that in females. Throughout the study period, FA concentration was kept constant in both males and females.	
Human Volunteer Male (20-27 years old) 13 persons	Dermal and inhalation exposure twice 4 hours	Dermal: 6.2 ppm (vapor) Inhalation: 7.1 ppm (vapor)	Absorption: Dermal absorption was 40.4% and respiratory absorption was 59.6%. Metabolism & excretion: Dermal: urinary NMF half-life: 4.75 hours Inhalation: urinary NMF half-life: 2.42 hours	Nomiyama et al., 2001
Human Resin plant worker Volunteer Male (20-39 years old) 3 persons	Exposure routes 1st: dermal and inhalation 2nd: dermal (with mask to protect inhale DMF) 8 hours/ exposure	1st : 16.7-40.0 ppm (vapor) 2nd : 12.2-35.2 ppm (vapor)	Absorption: Percentage[1)] of dermal absorption (3 persons[2)]): 62, 26, 27%, 1) Total is sum of dermal and pulmonary absorption 2) The 8-hour respiration volumes of the two persons with low dermal absorption rates were almost twice as much as that of the other person with high dermal absorption rate. Metabolism: Metabolized ratio to NMF in pulmonary absorption (3 persons): 2.1, 1.8, 4.1%	Miyauchi et al., 2001

<table>
<tr><th>Species</th><th>Route</th><th>Dose</th><th>Results</th><th>Reference</th></tr>
<tr><td>Rat
SD
Male and female
3-month old
About 10/ group</td><td>Intraperitoneal
Single or 4-day repeated dose</td><td>Single dose 949 mg/kg
(1 mL/kg)

4 days
475, 949 mg/kg/day (0.5, 1.0 mL/kg/day)</td><td>No dose-dependent increase of the metabolite, N-hydroxymethyl-N-methylformamide (DMF-OH).
Gender difference in excretion volume (higher in males), corresponding to increased plasma GDH and SDH activities indicating toxicity. The increase in enzymes was higher at a low dose and in males at the same dose.
DMF-OH excretion showed no correlation with dose, suggesting DMF-OH and other metabolites inhibit hydrolysis of N, N-dimethylformamide by themselves.

4-day administration
Changes of plasma enzyme activity at 24 hours after the last administration
(% to control)
<table>
<tr><th></th><th>Treated group</th><th>GDH[1)]</th><th>SDH[2)]</th></tr>
<tr><td rowspan="2">Male</td><td>475 mg/kg</td><td>590%</td><td>1,504%</td></tr>
<tr><td>949 mg/kg</td><td>—</td><td>872%</td></tr>
<tr><td rowspan="2">Female</td><td>475 mg/kg</td><td>—</td><td>791%</td></tr>
<tr><td>949 mg/kg</td><td>—</td><td>—</td></tr>
</table>
1) Glutamate dehydrogenase
2) Sorbitol dehydrogenase</td><td>Scailteur et al., 1984</td></tr>
</table>

<table>
<tr><th>Species</th><th>Route</th><th>Dose</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Human
Volunteer
10 persons

Mouse
BALB/c
Male

Rat
SD
Male

Hamster
Syrian
Male</td>
<td>Humans 10
Inhalation and
1 oral

Experimental
animal:
Intraperitonea
l</td>
<td>Human
Inhalation:
60 mg/m^3, 8 hours
(3.6 mg/mg
oral: 1.46, 7.31
mg/kg (0.02, 0.1
mM/kg)

Experimental
animal:
7.31, 51.17, 511.7
mg/kg (0.1, 0.7, 7
mM/kg)</td>
<td>Differences in urinary metabolites between animal species (% of dose)
<table>
<tr><th>Species
Dose</th><th>DMF[1)]</th><th>DMF-OH[2)]</th><th>NMF[3)]</th><th>AMCC[4)]</th></tr>
<tr><td>Human
(inhalation)
3.6 (mg/kg)</td><td>0.7</td><td>25.9</td><td>14.2</td><td>14.5</td></tr>
<tr><td>Mouse
511.7
511.7
7.31</td><td>
1.2
0.1
0</td><td>
45.5
18.2
8.4</td><td>
16.3
27.6
26.0</td><td>
1.1
1.3
1.6</td></tr>
<tr><td>Rat
511.7
511.7
7.31</td><td>
5.5
1.0
0</td><td>
44.6
43.2
36.8</td><td>
8.3
15.5
37.5</td><td>
1.7
2.7
5.2</td></tr>
<tr><td>Hamster
511.7
511.7
7.31</td><td>
2.2
0.3
0</td><td>
47.3
44.9
29.0</td><td>
7.9
24.0
22.9</td><td>
1.5
2.8
1.9</td></tr>
</table>
1): N, N-dimethylformamide
2): N-methylhydroxy-N-methylformamide (detected as methylformamide(NMF))
3): N-methylformamide (detected as formamide(FA))
4): N-acetyl-S-(N-methylcarbamoyl) cysteine

The percentage of AMCC was higher in humans than rodents.
The excretion rates of NMF and AMCC were high in mice and these metabolites were almost completely excreted within 24 hours. In rats, excretion rates were slow at the highest dose, and especially AMCC was not completely excreted within 24 hours. In hamsters, NMF was excreted early and AMCC excretion was slightly delayed at the highest dose.
With oral administration at doses of 1.46 and 7.31 mg/kg in humans, most of NMF was excreted within 12 hours, but AMCC was excreted slower and detected until 5 days after administration.
In humans, the excretion percentages of both NMF and AMCC were higher in inhalation exposure than those in oral administration.</td>
<td>Mraz et al., 1989</td>
</tr>
</table>

Species	Route	Dose	Results	Reference
Mouse CBA/CA Male	Intraperitoneal	^{14}C-labeled 6.8, 19.2 mmol/kg (497, 1,403 mg/kg)	Metabolism: 6.8 mmol/kg Within 24 hours after administration, 82.8% of radioactivity administered was excreted in the urine; 4.9% was unchanged *N, N*-dimethylformamide, 56.3% was C-hydroxylated and *N*-dimethylated derivatives, 3.4% was formamide and *N*-hydroxymethylformamide, and 18% was uncharacterized metabolites. The blood concentration of *N, N*-dimethylformamide rapidly increased and reached the peak at 4 hours after administration, and decreased thereafter. Metabolites DMF-OH and NMF were detected immediately after administration, their concentrations reached the peak at 8 hours after administration, and decreased thereafter, similarly to *N, N*-dimethylformamide.	Brindley et al., 1983
Mouse CBA/CA	Intraperitoneal administration	400 mg/kg	Metabolism: The major urinary metabolite was confirmed as DMF-OH with high resolution NMR and TCL/radio methods. Minimal dimethylamine and methylamine were detected by HPLC analysis.	Kestell et al., 1985
Rat Male SD	Intraperitoneal	1 mL/kg (100 μCi/mL)	Distribution: 24 hours after administration, approximately 4% of administered dose was detected in the blood and approximately 7% in other organs. Metabolism: in the urine, approximately 50% of administered dose was DMF-OH, 15% was unchanged *N, N*-dimethylformamide, approximately 5% was NMF-OH, other 20% was uncharacterized metabolites. Excretion: 90% of radioactivity administered was excreted in the urine within 72 hours. Other: Confirmation study whether NMF was *in vivo* product of DMF-OH or not. Dose: 1 mL/kg of MDF-OH 24 hours after administration, more than 50% of administered dose was excreted as unchanged DMF-OH. Within 72 hours, approximately 65% was excreted. NMF was not detected, suggesting that NMF was formed via a different metabolic pathway from DMF-OH. DMF-OH and NMF-OH generate formaldehyde in alkaline hydrolysis, therefore, DMF-treated rat urine was alkaline hydrolyzed and formaldehyde formation was confirmed. A product volume was almost the same as the NMF+F volume measured by analyzer. This result suggests that *N, N*-dimethylformamide is metabolized to DMF-OH and NMF-OH.	Scailteur et al., 1984

7.2 Epidemiological studies and case reports

The epidemiological studies and case reports of *N, N*-dimethylformamide are summarized in Table 7-2.

The acute effects of *N, N*-dimethylformamide on humans were observed in an accident that a plant worker was exposed to the skin due to spattering of *N, N*-dimethylformamide solution. The worker developed anorexia, vomiting and pain in the abdomen, hip and femor other than irritation to the skin, and after these symptoms dissapeared, fibril formation and histiocyte aggregation in the liver were observed (Potter, 1973). In addition to this accident case, some studies reported irritation to the eye, upper airway and gastrointestinal tract (Bainova, 1975; Kennedy, 1986;Tomasini et al., 1983). With regard to effects on the skin, dermatitis, eczema and vitiligo were reported (Bainova, 1975; Camarasa, 1987; Kennedy, 1986).

With regard to long-term exposure, many cases of hepatic dysfunctions due to occupational exposure have been reported. In biochemical examination, ALT, AST and γ-GTP were increased (Cirla et al., 1984; Fleming et al., 1990; Redlich et al., 1987; Wang et al., 1991; Wrbitzky, 1999), and diffuse hepatic degeneration and single cell necrosis were found in hepatic biopsy. Alcohol intolerance including hot flash and palpitation was reported (Cai et al., 1992; Lyle et al., 1979; Redlich et al., 1987; Tomasini et al., 1983; Wrbitzky, 1999), and these symptoms were considered due to inhibition of alcohol-metabolizing enzymes with *N, N*-dimethylformamide (Wrbitzky, 1999). Regarding genotoxicity of *N, N*-dimethylformamide, the population that was exposed to *N, N*-dimethylformamide showed increases in the incidences of chromosomal aberration and sister chromatid exchange in the peripheral lymphocytes (Major et al., 1998; Seiji et al., 1992).

With regard to carcinogenicity, testicular tumor developed in the workers of air plane repair plants (Ducatman et al., 1986) and leather tanning craftsmen (Levin et al., 1987) who used *N, N*-dimethylformamide. In the population of workers who were exposed to *N, N*-dimethylformamide in chemical plant, the incidences of oral and pharyngeal cancer were high (Chen et al., 1988). However, the causality between these cancer incidences and the exposure to *N, N*-dimethylformamide was unclear and these reports were considered not to be sufficient evidences of carcinogenicity (IARC, 1999).

At present, no reliable report on dose-response relationship of *N, N*-dimethylformamide exposure and carcinogenic effects in humans were obtained.

Table 7-2 Epidemiological studies and case reports of *N, N*-dimethylformamide

Population Gender/number	Exposure condition	Dose	Results	Reference
A worker in a fiber coating plant Male, 52 years old	Skin (20% of body surface) Inhalation exposure	Unknown	After exposure: Immediately: irritation to the skin, hyperemia 1 or 2 days: anorexia 62 hours: pain in the abdomen, hip and femor, vomiting 11 days: The observed clinical symptoms disappeared, but fibril formation and histiocyte aggregation were observed in the liver.	Potter, 1973
Workers in a artificial leather plant, 14 subjects	Unknown	14 -60 mg/m^3	Irritation to the eye, upper airway and gastrointestinal tract, alcohol intolerance	Tomasini et al., 1983
Workers in a polyacrylonitrile fiber plant	Unknown	30-60 ppm	Fatigue, weakness, peripheral numbness, irritation to the eye and throat	Kennedy, 1986

Population Gender/number	Exposure condition	Dose	Results	Reference
A worker in an epoxy resin plant Female, 19 years old	Several months	Unknown	Severe contact dermatitis associated with itch on the back and side of both hands Patch test: positive	Camarasa, 1987
Unknown	Unknown	Unknown	Skin sensitivity, allergic dermatitis, eczema and vitiligo	Bainova, 1975; Kennedy, 1986
Workers using *N, N*-dimethylformamide 5/5 persons	Unknown	Unknown	Pain in the upper abdomen, fever in the back, nausea, vomiting, rush and itch in the palm and forearm, pain in the epigastrium, an increase in serum amylase, the possible pancreatitis	Chary, 1974
Workers Male 19/102 persons	Occupational exposure	16-200 ppm	Hot flash (especially alcohol intake)	Lyle et al., 1979
Acrylic fiber plant	Unknown	Unknown	Esophagitis, gastritis, hepatitis	Guirguis, 1981
Workers in polyurethane plant, 100 persons	Unknown	22 mg/m^3 5 years	Headache, dyspepsia, liver and gastrointestinal disorder, an increase in γ-GTP	Cirla et al., 1984
An operator of fiber coating machine Male, 40 years old		2 weeks work	Abdominal pain, nausea, headache, an increase in AST 3.5 months after reassignment, an increase in ALT, diffuse degeneration, change in hepatocellular nuclear size, binucleate cells, single cell necrosis, Kupffer cell hypertrophy in hepatic biopsy	Redlich et al., 1988
Workers in a fiber coating plant	Insufficient ventilation and skin protect	Unknown	Increase in hepatic disorder Increase in AST or ALT (36/46), anorexia, abdominal pain or nausea (31/46), headache and dizziness (18/46), alcohol intolerance (hot flash and palpitation) (11/46)	Redlich et al., 1987
Workers	Unknown	Unknown	Focal hepatic necrosis, fatty degeneration of microvesicle in the smooth endoplasmic reticulum, polytypic mitochondria associated with crystallin inclusion No incidence of liver fibrosis	Redlich et al., 1990
Workers: 45 Control: 12	Unknown	Unknown	Increases in serum ALT and AST	Fleming et al., 1990
Workers in at an artificial leather plant 204 persons	Unknown	25-60 ppm	Increases in serum ALT and creatinine phosphokinase Dizziness, anorexia, nausea, pain in the upper abdomen	Wang et al., 1991
Workers: 318 Control: 143	Unknown	7 ppm (21 mg/m^3) and above	Dose-dependent nausea and abdominal pain Increase in alcohol intolerance No hematological and biochemical changes	Cai et al., 1992
Workers at a synthetic fiber plant, mean 42 years old 126 males	Unknown	0.1-37.9 ppm (mean 1.2 ppm)	Increases in γ-GTP and AST activities, hot flash after drinking (exposed: 69.9%, non-exposed: 3.8%) Enhanced alcohol hepatotoxicity due to inhibition of alcohol and aldehyde dehydrogenase Increase in sensitivity to *N, N*-dimethylformamide induced by hepatitis B	Wrbitzky, 1999
Unknown	Unknown	Unknown	Abnormal cardiovascular system, neuropathy	Aldyreva et al., 1980

Population Gender/number	Exposure condition	Dose	Results	Reference
U.S.A. White male Jet plane repair plant (3 sites)	Use of 80%-*N*, *N*-dimethylformamide solvent (Plants A and B)	Unknown	Development of testicular tumors Plant A: testicular germ cell tumors developed in 3 of 153 workers from 1981 to 1983 Plant B: testicular germ cell tumors developed in 4 of 680 workers from 1970 to 1983 Plant C: no testicular germ cell tumor developed in all of 446 workers. Pathological diagnoses of tumors were disgerminoma in 5 persons and embryonal cell cancer in 2 persons.	Ducatman et al., 1986
U.S.A. leather tanning craftsman	Other than *N*, *N*-dimethylformamide, various kinds of solvent and dyestuff were used.	Unknown	Development of testicular tumors embryonal cell cancer in 3 persons	Levin et al., 1987
DuPont employees: 2,530 workers who were exposed to *N*, *N*-dimethylformamide in Virginia Plant from 1950 to 1970 1,329 workers who were exposed to DMF and acrylonitrile in South California Plant		Unknown	Standardized Incidence Rate (SIR) of all cancers: 1.1 (88 persons) One person developed testicular cancer (expected value: 1.7) Mortality incidences of oral and esophageal cancers increased in workers who were exposed to *N*, *N*-dimethylformamide (1950-1982)	Chen et al., 1988
Human Peripheral lymphocyte 40 persons	Exposed to minimal methylethylketone, butyl acetate, toluene, cyclohexanone, xylene other than *N*, *N*-dimethylformamide	[1]180 mg/m^3 [2]150 mg/m^3 (1 month after) [3] 50 mg/m^3 (6 months thereafter) [4] 40 mg/m^3 (6 months thereafter) [5] 35 mg/m^3 (6 months thereafter)	Chromosome aberration Control group: 1.10-1.61% Exposed group: [1] 180 mg/m^3 3.82% [2] 150 mg/m^3 2.74% [3] 50 mg/m^3 1.59% [4] 40 mg/m^3 1.58% [5] 35 mg/m^3 1.49%	Koudela & Spazier, 1981
Human Peripheral lymphocyte 20 persons	Unknown	*N*, *N*-dimethylformamide: 12.3 mg/m^3 Monomethylformamide: 5.3 mg/m^3 Dimethylamine: 0.63 mg/m^3	Chromosome aberration The incidences of chromosomal gaps and breaks were 0.4% in the control group and 1.4% in the exposed group. (control group: 18 non-exposed workers in the same plant)	Berger et al., 1985

Population Gender/number	Exposure condition	Dose	Results	Reference
Human Female 22 persons	Occupational exposure to DMF	High exposure group: 5.8 ppm (17.4 mg/m^3) Middle exposure group: 0.7 ppm (2.1 mg/m^3) Low exposure group: 0.3 ppm (0.9 mg/m^3)	Incidence of sister chromatid exchange per peripheral lymphocyte (%) Control Exposure High concentration 5.63 8.26 Middle concentration 4.66 7.24 Low concentration 6.57 5.67 The incidences in the high and middle exposure groups were higher than that in the low exposure group.	Seiji et al., 1992
Workers at a viscose and rayon plant (mean 33 years, 26 males, maintenance: 13, manufacturing: 13)	Chronic exposure 3-10 years	Unknown The peak concentration in the air during study period was 0.6-23.0 mg/m^3 at the initiation of study, 7 months after: 3.5 - 22.8 mg/m^3	Hematological effects including hepatic dysfunction and increase in lymphocytes Increases in the incidences of chromosomal aberration, sister chromatid exchange and unscheduled DNA synthesis in the peripheral lymphocytes The incidence of sister chromatid exchange was higher in workers of manufacturing division (2.72 fold to that of maintenance division).	Major et al., 1998

7.3 Studies in experimental animals and *in vitro* studies

7.3.1 Acute toxicity

Acute toxicity data of *N, N*-dimethylformamide to experimental animals are shown in Table 7-3.

The acute toxicity of *N, N*-dimethylformamide to experimental animals with oral administration was relatively low, and the LD_{50} was 3,700 to 6,800 mg/kg in mice and 2,000 to 7,600 mg/kg in rats. The LC_{50} for inhalation exposure was 2,000 to 6,120 ppm in mice and 2,500 to 5,020 ppm in rats. It was reported that the toxicity was found more frequently in juvenile animals (Kimura et al.,1971). Toxicity symptoms observed included body weight loss, restlessness, sensitiveness, liver necrosis and congestion, lung congestion and edema, renal tubular epithelium swelling, myelosuppression, and alveolar wall thickening in an oral administration study in rats (E.I. Dupont de Nemours & Co., 1970a). In an intraperitoneal administration study in mice, sedation and hind-limb paralysis following restlessness were observed (Davis and Jenner, 1959), and in rats liver congestion, a decrease in glycogen, central vein phlebitis, hepatocyte anisokaryosis (heteromorphism) and centrilobular hepatic necrosis (Mathew et al., 1980).

Table 7-3 Acute toxicity of *N, N*-dimethylformamide

	Mouse	Rat	Guinea pig	Gerbil	Rabbit	Cat	Dog
Oral LD_{50} (mg/kg)	3,700-6,800	2,000-7,600	3,400	3,000-4,000	>5,000	ND	ND
Inhalation LC_{50} (ppm)	2,000-6,120	2,500-5,020	ND	ND	ND	ND	ND
Dermal LD_{50} (mg/kg)	5,000-11,000	>11,520	ND	ND	500-1,500	ND	ND
Intraperitoneal LD_{50} (mg/kg)	300-6,200	1,400-5,470	1,300-4,000	3,000-4,000	945-5,000	300-500	ND
Intravenous LD_{50} (mg/kg)	2,500-4,100	2,000-3,000	1,000-1,030	ND	1,000-1,800	ND	470-500

	Mouse	Rat	Guinea pig	Gerbil	Rabbit	Cat	Dog
Subcutaneous LD_{50} (mg/kg)	3,500-6,500	3,500-5,000	ND	3,000-4,000	2,000	ND	ND
Intramuscular LD_{50} (mg/kg)	3,800-6,500	4,030	ND	ND	ND	ND	ND

ND: No data available

7.3.2 Irritation and corrosion

Studies on the irritation and corrosion of *N, N*-dimethylformamide to experimenal animals are summarized in Table 7-4.

The irritation of *N, N*-dimethylformamide to eyes was investigated in rabbits (E.I. DuPont de Nemours & Co., 1970b; Massmann, 1956; Williams et al., 1982).

After application of *N, N*-dimethylformamide to the eyes, a rabbit without eye-washing showed moderate corneal damage, slight to moderate conjunctival hyperemia and slight swelling, and slight to moderate lacrimation, but the iris was not affected. In contrast, a rabbit with eye-washing developed moderate to severe corneal damage and distortion and angiogenesis around the cornea, in addition, slight iritis and slight to moderate conjunctival hyperemia, swelling and lacrimation. It was reported that the stronger reactions were observed in the rabbit with eye-washing.

In a dermal irritation study of *N, N*-dimethylformamide in mice at doses of 1,000, 2,500 and 5,000 mg/kg, slight transient irritation was observed at doses of 2,500 mg/kg and above (Wiles and Narcisse, 1971). In dermal application of *N, N*-dimethylformamide to rabbits at doses of 100, 250 and 500 mg/kg, no irritation was observed (Wiles and Narcisse, 1971). Also in a study at a dose of 2,000 mg/kg for 15 days/4 weeks (6 hours/day), no dermal irritation was found in rabbits (Kennedy, 1986). In dermal application studies in rats and guinea pigs at single dose, no irritation was observed (Kiss, 1979).

Table 7-4 Irritation and corrosion of *N, N*-dimethylformamide

Species	Test method Route	Period	Dose	Results	Reference
Rabbit	Instillation	Unknown	0.01 mL	Cornea: moderate damage Conjunctiva: moderate to severe conjunctivitis	Massmann, 1956; Williams et al., 1982
Rabbit	Instillation in the conjunctival sac	Unknown	25, 50, 75, 100 g/L	25 g/L: no effect 50 g/L: slight irritation 75 and 100 g/L: severe irritation	Massmann, 1956
Rabbit NZW 2 males age unknown	Instillation	Once	0.1 mL	Unwashed Cornea: moderate damage Iris: no effect Conjunctiva: slight to moderate hyperemia slight swelling slight to moderate lacrimation Washed Cornea: moderate to severe damage angiogenesis around the cornea distortion (1 site) Iris: slight iritis Conjunctiva: slight to moderate hyperemia slight to moderate swelling slight to moderate lacrimation	E.I. DuPont de Nemours & Co., 1970b

Species	Test method Route	Period	Dose	Results	Reference
Mouse	Dermal application	Unknown	1,000, 2,500, 5,000 mg/kg	At 2,500 mg/kg and above: slight transient irritation	Wiles & Narcisse, 1971
Mouse	Dermal application	2-3 hours	500, 2,500 mg/kg	Slight dermal irritation	Wiles & Narcisse, 1971
Rat	Dermal application	Single dose	Unknown	No dermal irritation	Kiss, 1979
Guinea pig	Dermal application	Single dose	Unknown	No dermal irritation	Kiss, 1979
Rabbit	Dermal application	Unknown	100, 250, 500 mg/kg	No dermal irritation	Wiles & Narcisse, 1971
Rabbit	Dermal application	6 hours/day 15 days/4 weeks	2,000 mg/kg	No dermal irritation	Kennedy, 1986

7.3.3 Sensitization

No reports of sensitization studies in experimental animals were obtained in this investigation.

7.3.4 Repeated dose toxicity

Studies on the repeated dose toxicity of *N, N*-dimethylformamide to experimental animals are summarized in Table 7-5.

a. Oral administration

Male and female mice were fed diet containing *N, N*-dimethylformamide at concentrations of 0, 160, 540 and 1,850 ppm (corresponding to 0, 22, 70 and 246 mg/kg/day in males and 0, 28, 96 and 326 mg/kg/day in females) for 119 days. An increase in relative liver weight was observed in females at concentrations of 540 ppm and above. No histopathological changes were found, and therefore, this increase was considered to be an adaptive change (Becci et al., 1983).

Rats were orally administrerd by gavage at doses of 0 and 450 mg/kg/day for 2 weeks. Suppression of body weight gain, anisokaryosis, an increase in mitotic index and binucleate cells in hepatocytes were found. All of them recovered in the 11-day recovery period (Kennedy and Sherman, 1986).

In oral administration (via drinking water or via diet) studies in rats, an increase in liver weight was commonly found (Becci et al., 1983; Qin and Gue, 1976; U.S. EPA, 1986).

Male and female SD rats were fed diet containing *N, N*-dimethylformamide at concentrations of 0, 200, 1,000 and 5,000 ppm (corresponding to 0, 17.2, 86.2 and 431 mg/kg/day) for 90 days. Hypercholesterolemia was observed at concentrations of 1,000 ppm and above, and an increase in mitotic figure and hypertrophy in hepatocytes at 5,000 ppm (Kennedy and Sherman, 1986; U.S. EPA, 1986). The NOAEL of this study is considered to be 200 ppm (corresponding to 17.2 mg/kg/day) in this assessment.

In a oral administration (via drinking water) study in gerbils at concentrations of 0, 10,000, 17,000, 34,000 and 66,000 ppm for 200 days, death was observed at 10,000 ppm and above and all animals at 17,000 ppm and above died by 80 days after administration. At 17,000 ppm and above, histopathological liver changes including diffuse necrosis, an increase in mitotic figure, karyomegaly, hemosiderosis and an

increase in Kupffer cells in hepatocytes were found, and kidney congestion was observed at 17,000 ppm (Llewellyn et al., 1974).

In an oral feeding study of *N, N*-dimethylformamide in dogs (4 animals) at a dose of 25 mg/kg/day for 10 weeks and at a dose of 50 mg/kg/day for additional 2 weeks, transient changes in cardiac function was observed, however, effects on organs were not reported (U.S. EPA, 1986).

b. Inhalation exposure

In a whole body inhalation study of *N, N*-dimethylformamide in male and female BDF_1 mice at concentrations of 0, 100, 200, 400, 800 and 1,600 ppm for 2 weeks (6 hours/day, 5 days/week), no animals died in males and females at all concentrations. In males, centrilobular hepatocyte degeneration (associated with glycogen depletion and basophilic changes) was observed at 200 ppm and above, an increase in relative liver weight at 400 ppm and above, and suppression of body weigh gain, focal hepatic necrosis, centrilobular single cell necrosis associated with fragmented nucleoli at 1,600 ppm. In females, an increase in relative liver weight was observed at 200 ppm and above, centrilobular hepatocyte degeneration (associated with glycogen depletione and basophilia) at 800 ppm and above, and suppression of body weigh gain, focal necrosis in hepatocyte, centrilobular single cell necrosis associated with fragmented nucleoli at 1,600 ppm (Senoh et al., 2003).

In a inhalation exposure study of *N, N*-dimethylformamide in male and female $B6C3F_1$ mice at concentrations of 0, 50, 100, 200, 400 and 800 ppm for 13 weeks (6 hours/day, 5 days/week), an increase in liver weight, centrilobular hepatocyte hypertrophy and necrosis were found at 50 ppm (152 mg/m^3) and above. A prolonged estrous cycle was observed at 200 ppm (600 mg/m^3) and above (Lynch et al., 1991; U.S. NTP, 1992). The LOAEL of this study is considered to be 50 ppm in this assessment.

In a whole body inhalation exposure study of *N, N*-dimethylformamide in male and female BDF_1 mice at concentrations of 0, 50, 100, 200, 400 and 800 ppm for 13 weeks (6 hours/day, 5 days/week), no animals died in males and females at all concentration. In males, suppression of body weight gain, increases in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and relative liver weight (absolute weight was also increased but not dose-dependently), and centrilobular hepatocyte degeneration were found at 50 ppm and above, an increase in platelet count at 50 and 100 ppm, focal hepatic necrosis (associated with ceroid and hemosiderin) at 100 ppm and above, an increase in total cholesterol at 100 and 400 ppm, and a reduction in feed consumption, an increase tendency in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities, massive hepatic necrosis (3 animals), single cell necrosis in hepatocytes (associated with fragmented nucleoli), and centrilobular hepatocyte hypertrophy at 800 ppm. In females, increases in MCV and MCH, total cholesterol, and a dose-independent increase in absolute liver weight were found at 50 ppm and above, an increase in ALP activity at 100 ppm and above, an increase in ALT activity at 200 ppm and above, and an increasing tendency of AST activity, increases in LDH activity and serum urea nitrogen (BUN), hepatic single cell necrosis (associated with fragmented nucleoli), and centrilobular hepatocyte hypertrophy at 800 ppm (Senoh et al., 2003). At 50 ppm, the lowest exposure concentration, suppression of body weight gain (male), increases in mean corpuscular volume (male and female), mean corpuscular hemoglobin (male and female)

and relative liver weight (male), and centrilobular hepatocyte hypertrophy (male) were found. The LOAEL of this study is is considered to be 50 ppm (151 mg/m^3) in this assessment.

An inhalation exposure study of *N, N*-dimethylformamide in ICR mice at concentrations of 0, 25, 100 and 400 ppm for 18 months (6 hours/day, 5 days/week) was carried out. The hepatic hypertrophy and single cell necrosis, lipofuscin and hemosiderin pigment in Kupffer cells were found at 25 ppm (76 mg/m^3) and above, and an increase in liver weight at 100 ppm (304 mg/m^3) and above. The authors reported the NOAEL as less than 25 ppm (76 mg/m^3) (Malley et al., 1994).

In a whole body inhalation exposure study of *N, N*-dimethylformamide in male and female BDF_1 mice at concentrations of 0, 200, 400 and 800 ppm for 104 weeks (6 hours/day, 5 days/week), the body weight gain was dose-dependently suppressed in males and females at all concentrations, and at the end of study period, body weight of males at all concentrations and females at 800 ppm was less than 10% of body weight of the control animals. The food consumption of males and females at all concentrations were almost similar to that of the control animals and no abnormal changes were found in clinical observations. The survival rate of females at 800 ppm was lower than that of the control group due to development of liver tumors in 78 weeks and later. Survival rates in other treated groups were almost similar to that of the control group. In blood biochemistry, increases in serum AST, ALT, γ-GTP, ALP and creatine phosphokinase (CPK) activities, total serum protein and cholesterol, and relative and absolute liver weight were found in males at 200 ppm and above, and increases in serum albumin and BUN at 400 ppm and above, and an increase in serum total bilirubin at 800 ppm. In females, increases in serum AST, ALT, γ-GTP, ALP and CPK activities, total serum bilirubin, protein, albumin and cholesterol, and BUN were found at 200 ppm and above. In males and females at 200 ppm and above, increases in relative and absolute liver weight were observed. In macroscopic pathological examination, red or reddish brown nodules of the liver were observed in males and females at 200 ppm and above. In histopathological examination, in addition to development of neoplastic lesions including hepatocyte adenoma, an increase in small foci of hepatocytes, pre-neoplastic lesions were observed (see Section 7.3.7). In males, centrilobular hepatocyte hypertrophy and dyskaryosis, single cell necrosis and an increase in inflammatory cells in the liver were found at all concentrations, and in females, centrilobular hepatocytes hypertrophy at 200 and 800 ppm and an increase in centrilobular dyskaryosis at 800 ppm. No histopathological change was observed in organs other than the liver (Senoh et al., 2004).

In a whole body inhalation exposure study of *N, N*-dimethylformamide in male and female F344 rats at concentrations of 0, 100, 200, 400, 800 and 1,600 ppm for 2 weeks (6 hours/day, 5 days/week), suppression of body weight gain was found in females at 400 ppm and above, single cell necrosis associated with nucleolar plasmotomy in the liver in males and females at 800 ppm, and suppression of body weight gain in males at 800 ppm and above. At 1,600 ppm, 3 males and 7 females died and an increase in relative liver weight was observed in males and females, and massive necrosis associated with hemorrhage, congestion, fibril formation and focal calcification of marked hepatic centrilobular cells were found in dead animals (Senoh et al., 2003).

In a inhalation exposure study of *N, N*-dimethylformamide in rats at a concentration of 200 ppm (600 mg/m^3) for 28 days (8 hours/day), increases in serum ALT and AST were found (Tanaka, 1971).

A whole body inhalation study of *N, N*-dimethylformamide in F344 rats at concentrations of 0, 50, 100, 200, 400 and 800 ppm (corresponding to 0, 152, 304, 608, 1,216 and 2,436 mg/m^3) for 13 weeks (6 hours/day, 5 days/week) was carried out. An increase in cholesterol, a decrease in total protein and an increase in liver weight were observed at 50 ppm and above, a decrease in MCH and an increase in platelet count at 100 ppm and above, decreases in MCV, ALP and albumin and an increase in sorbitol dehydrogenase (SDH) at 200 ppm and above, increases in red blood cells, total bilirubin and ALT, a decrease in total protein, a change in albumin, centriacinar hepatic necrosis at 400 ppm and above, and increases in hematocrit, hemoglobin and isocitrate dehydrogenase and prolonged estrous cycle at 800 ppm and above. Based on centriacinar hepatic necrosis observed only at 400 ppm and above, the authors reported the NOAEL as 200 ppm (608 mg/m^3) with the endpoint of histopathological changes (Lynch et al., 1991; U.S. NTP, 1992). However, the NOAEL of this study is considered to be 50 ppm (152 mg/m^3) in this assessment.

An inhalation exposure study of *N, N*-dimethylformamide in SD rats at concentrations of 0, 25, 100 and 400 ppm (0, 76, 304, 1,216 mg/m^3) for 2 years (6 hours/day, 5 days/week) was carried out. The suppression of body weight gain, increases in liver weight and centrilobular hepatocyte hypertrophy were observed at 100 ppm and above, single cell necrosis and centrilobular lipofuscin and hemosiderin pigment at 400 ppm. The NOEL was considered to be 25 ppm (76 mg/m^3) (Malley et al., 1994).

In a whole body inhalation exposure study of *N, N*-dimethylformamide in male and female F344 rats at concentrations of 0, 50, 100, 200, 400 and 800 ppm for 13 weeks (6 hours/day, 5 days/week), increases in total cholesterol and phospholipid were found in males at 50 ppm and above, an increase in relative liver weight at 100 ppm and above, hepatocytes necrosis (sometimes associated with ceroid and hemosiderin, and nucleolar plasmotomy and cell division images) at 200 ppm and above, suppression of body weight gain and centrilobular hepatocyte hypertrophy at 400 ppm and above, and a reduction in feed consumption, increases in AST, ALT and LDH activities, a decrease in triglyceride and an increase in total bilirubin at 800 ppm. In females, an increase in phospholipid was observed at 100 ppm and above, increases in total cholesterol, triglyceride and relative liver weight, hepatic single cell necrosis (sometimes associated with ceroid and hemosiderin, and nucleolar plasmotomy and cell division images) at 200 ppm and above, suppression of body weight gain, increases in ALT and γ-GTP activities and total bilirubin and centrilobular hepatocyte hypertrophy at 400 ppm and above, and a reduction in food consumption, increases in AST and LDH activities and massive hepatolobular necrosis (one animal) at 800 ppm (Senoh et al., 2003).

In a whole body inhalation exposure study of *N, N*-dimethylformamide in male and female F344 rats at concentrations of 0, 200, 400 and 800 ppm for 104 weeks (6 hours/day, 5 days/week), animals died in early stage of exposure. The body weight gain was dose-dependently suppressed in males and females at all concentrations, and at the end of study period, body weight of males and females at 400 and 800 ppm was less than 90% of body weight of the control animals. Food consumption was reduced in females at 800 ppm. At all concentrations, no abnormal changes were observed in clinical observations. No significant difference in survival rate was found between the controls and males at all concentrations and females at 200 ppm, however, the survival rate of females at 800 ppm was decreased at 9 weeks after exposure and the cause of death in 21 weeks after exposure was centrilobular necrosis associated with red spot or clarified

hepatic lobule (macroscopic observation). In blood biochemistry, increases in AST, ALT, γ-GTP and ALP activities, total bilirubin, total cholesterol, phospholipids and blood BUN were increases in males at 200 ppm and above, and an increase in LDH activity at 800 ppm. In females, increases in total bilirubin, total cholesterol and phopholipid were increased at 200 ppm and above, and an increase in ALP activity at 400 ppm and above. In macroscopic observation at necropsy, males and females that were exposed to *N, N*-dimethylformamide at 800 ppm and survived for 2-year showed white or brown nodes in the liver. In males and females at 200 ppm and above, increases in relative and absolute liver weight (excluding 400 ppm) were found. In histopathological examination, dead males and females showed centrilobular hepatic necrosis, especially the animals died early in the study had severe lesions. Increases in development of hepatocellular carcinoma and adenoma were found in males and females, and an increase in altered cell foci in hepatocytes, a pre-neoplastic lesions were observed (see Section 7.3.7). No histopathological change was observed in organs other than the liver (Senoh et al., 2004).

In an inhalation exposure study in rabbits at 0, 40 ppm (0, 122 mg/m^3) for 50 days showed a myocardial change (Arena et al., 1982). In an inhalation exposure study in dogs at a concentration of 50 ppm (152 mg/m^3) for 3 weeks, a change in blood pressure was found (U.S. EPA, 1986).

Inhalation exposure study of *N, N*-dimethylformamide in cynomolgus monkeys at a concentration of 500 ppm (1,520 mg/m^3) for 2 weeks (6 hours/day, 5 days/week) and a inhalation exposure study at concentrations of 0, 30 to 500 ppm (91 to 1,520 mg/m^3) for 13 weeks (6 hours/day, 5 days/week) were conducted, but no effects were found (Hurrt et al., 1991).

Combination of exposures at a low concentration and a short-term high concentration. Rats were exposed to *N, N*-dimethylformamide at a concentration of 0, 91 ppm (277 mg/m^3) for 10 days (6 hours/day) [1], 1,104 ppm (3,356 mg/m^3) for 10 days (0.5 hours/day) [2], or 91 ppm (corresponding to 277 mg/m^3) for 10 days (6 hours/day) and subsequent 841 ppm (2,557 mg/m^3) for one day (0.5 hours/day) [3]. In all dosage patterns, relative liver weight was increased, however, the toxic effect was involved in the product of exposure concentration and time, i.e., the results were similar between [1] and [2] but the result of [3], combination [1] and a single exposure at high concentration, was higher than that of [1]. Based on the result, the authors indicated that a short-term exposure of *N, N*-dimethylformamide at high concentration induces the same damage as induced by a long-term exposure at low concentrations (Clayton et al., 1963).

c. Dermal application

In the study in rats at a dose of 0, 474.5 mg/kg/day for 1 week, increases in ATP, AST, ALP, γ-GTP and cholinesterase and decreases in hepatic P450 and glutathione reductase activity were found (Imazu et al., 1992).

In dermal application study of *N, N*-dimethylformamide in rabbits and guinea pigs at a dose of 2 mL of undiluted *N, N*-dimethylformamide for 7 days (3 times/day) (Huang et al., 1981) and dermal apllication study in rabbits at a dose of 2,000 mg/kg/day for 2 weeks (Kennedy and Sherman, 1986), some of animals died. In dermal application studies in rats for 28 to 30 days, ATP, AST, ALP, γ-GTP and cholinesterase were dose-dependently increased (Bainova and Antov, 1980; Bainova et al., 1981). Regarding effects on general conditions, in dermal application study in rabbits at a dose of 2,000 mg/kg/day for 2 weeks, body weight

loss, anorexia, weakness and cyanosis, and hepatic congestion and necrosis were found (Kennedy and Sherman, 1986).

As described above, repeated administration of *N, N*-dimethylformamide had effects on the liver in all administration routes and common effects are the increase of liver weight, hepatic degeneration and necrosis and biochemical changes. Other than the liver, renal disorder and changes in cardiac function and myocardium were found and the U.S. NTP repoted prolonged estrous cycle in mice and rats in a 13-week inhalation exposure study.

Based on these results, the NOAEL of *N, N*-dimethylformamide for oral administration is considered to be 200 ppm (corresponding to 17.2 mg/kg/day) with the results of the 90-day oral (feeding) study of *N, N*-dimethylformamide in rats (Kennedy and Sherman, 1986; U.S. EPA, 1986), and the LOAEL for inhalation exposure as 25 ppm (76 mg/m^3) with the results of the 18-month inhalation exposure study in mice (Malley et al., 1994) and the NOAEL as 25 ppm (76 mg/m^3) with the results of the 2-year inhalation exposure study in rats (Malley et al., 1994).

Table 7-5 Repeated dose toxicity of *N, N*-dimethylformamide

Species sex/number of animals	Route	Period	Dose	Results	Reference
Mouse ICR Male and female 6 to 8 weeks	Oral Feeding	119 days	0, 160, 540, 1,850 ppm (Male: corresponding to 0, 22, 70, 246 mg/kg/day; Female: corresponding to 0, 28, 96, 326 mg/kg/day)	540 ppm: Male: No effect Female: increase in the relative liver weight 2,500 ppm: Male and female: increases in absolute and relative liver weight	Becci et al., 1983
Rat SD Male young adult 6 animals /group	Oral gavage	9 times/ 2 weeks (recovery in 11 days)	0, 450 mg/kg/day	450 mg/kg/day (during administration period and at the end of study period): suppression of body weight gain, an increase in feed consumption, transient restlessness, hepatocyte anisokaryosis, an increase in mitosis, binucleate cells Recovery group: No effect on body weight gain, no histopathological change in the liver	Kennedy & Sherman, 1986
Rat	Oral Feeding	30 days	0, 320, 640 ppm	Anorexia, body weight loss	Qin & Gue, 1976
Rat SD Male and female young adult 6 animals /group	Oral Feeding	90 days	0, 200, 1,000, 5,000 ppm (corresponding to 0, 17.2, 86.2, 431 mg/kg/day, in this	Male: 1,000 ppm and above: hypercholesterolemia, a decrease in liver fat, an increase in relative liver weight 5,000 ppm: suppression of body weight gain, low feed consumption, slight anemia, increases in white blood cells (WBC) and mitotic figures, slight hypertrophy of hepatocytes Female:	U.S. EPA, 1986; Kennedy & Sherman, 1986

Species sex/number of animals	Route	Period	Dose	Results	Reference
			assessment)	1,000 ppm and above: hypercholesterolemia associated with hepatic adipose loss, an increase in relative liver weight 5,000 ppm: suppression of body weight gain, low food consumption, slight anemia, increases in WBC and mitotic figures, slight hypertrophy of hepatocytes NOAEL:200 ppm (corresponding to 17.2 mg/kg/day) (in this assessment)	
Rat	Drinking water	100 days	0, 50, 500, 5,000 ppm	500 ppm and above: body weight loss, an increase in relative liver weight 5,000 ppm: hepatic damage and degeneration NOAEL : 50 ppm	Qin & Gue, 1976
Rat Wistar Male and female 6 to 8 weeks	Oral Feeding	104 days	0, 215, 750, 2,500 ppm	750 ppm and above: Female: low food consumption 2,500 ppm: Male: low feed consumption, suppression of body weight gain, an increase in relative liver weight Female: suppression of body weight gain, increases in absolute and relative liver weight	Becci et al. 1983
Gerbil Female 12 animals /group	Drinking water	200 days	0, 10,000, 17,000, 34,000, 66,000 ppm	10,000 ppm: 25% died by the end of administration period 17,000 ppm: all died by administration days 22 to 80. 34,000 ppm: all died by administration days 3 to 19. 66,000 ppm: all died by administration days 1 to 22, body weight loss Histopathological change: 10,000 ppm and above: hepatic necrosis foci (dead animal) 17,000 ppm and above: diffuse hepatic necrosis, increases in hepatocyte nuclear stain and mitosis, giant nuclei, hemosiderosis and an increase in Kupffer cells, kidney congestion	Llewe-llyn et al., 1974
Dog 4 animals	Oral Feeding	12 weeks	25mg/kg/day 10 weeks (5days/ week) 50mg/kg/day 2 weeks (5 days/ week)	Transient change in cardiac function. No effect on blood pressure and organs	U.S. EPA, 1986
Mouse BDF_1 Male and female 6 weeks 10 animals /group	Inhalation according to the OECD test guideline 412	2 weeks (6 hours/day 5 days/ week)	0, 100, 200, 400, 800, 1600 ppm (0, 304, 608, 1216, 2432, 4864 mg/m^3) mean actual concentra-tion: 0, 101.1, 203.6,	Male and female: No dead animal Male: 200 ppm and above: centrilobular hepatocyte degeneration (associated with glycogen shortage and basification) 400 ppm and above: an increase in relative liver weight 1,600 ppm: suppression of body weigh gain, focal hepatocytes necrosis (associated with inflammatory cess infiltration), centrilobular single cell necrosis associated with nucleolar plasmotomy	Senoh et al., 2003

Species sex/number of animals	Route	Period	Dose	Results	Reference
			407.9, 806.6, 1623.8 ppm (0, 307, 619, 1,240, 2,452, 4,936 mg/m^3)	Female: 200 ppm and above: an increase in relative liver weight Male: centrilobular hepatocyte degeneration (associated with glycogen shortage and basification) 800 ppm and above: centrilobular hepatocyte degeneration (associated with glycogen shortage and basification) 1,600 ppm: suppression of body weigh gain, focal hepatocytes necrosis (associated with inflammatory cess infiltration), centrilobular single cell necrosis associated with nucleolar plasmotomy	
Mouse Male age unknown 11 animals	Inhalation	58 times (6 hours/day 5 days/ week)	0, 23 ppm× 5.5 hours + 426 ppm× 0.5 hours	23 ppm: No dead animal Male: increase in liver weight	Clayton et al., 1963
Mouse B6C3F$_1$ Male and female Young adult	Inhalation	12 weeks (6 hours/day 5 days/ week)	0, 150, 300, 600, 1,200 ppm mean actual concentra-tion: 0, 0.817, 148.6, 302.4, 587.3, 1,184 ppm (0, 452, 919, 1,785, 3,599 mg/m^3)	Death: 600 ppm: 2 animals, 1,200 ppm: 8 animals Male: 150 ppm and above: centrilobular hepatocyte hypertrophy 300 ppm: hepatic necrosis 600 ppm: hepatic discoloration or degeneration 600 ppm and above: hepatic necrosis, yellow-brown pigment in Kupffer cells Female: 150 ppm: hepatic necrosis 150 ppm and above: centrilobular hepatocyte hypertrophy 600 ppm and above: hepatic discoloration or degeneration, necrosis, yellow-brown pigment in Kupffer cells and phagocytes LOAEL: 150 ppm	Craig et al., 1984
Mouse B6C3F$_1$ Male and female 46 day old	Inhalation	13 weeks (6 hours/day 5 days/ week)	0, 50, 100, 200, 400, 800 ppm (0, 152, 304, 608, 1,216, 2,432 mg/m^3)	Male: 50 ppm and above: an increase in relative liver weight, centrilobular hepatocyte necrosis and hypertrophy 200 ppm and above: an increase in absolute liver weight 400 ppm and above: brown liver Female: 50 ppm and above: increases in absolute and relative liver weight, centrilobular hepatic necrosis 100 ppm and above: centrilobular hepatocyte hypertrophy 200 ppm and above: prolonged estrous cycle NOAEL : Female: 50 ppm Male: not determined (author) LOAEL : 50 ppm (in this assessment)	Lynch et al., 1991; Lynch et al., 2003; U.S.NTP, 1992
Mouse BDF$_1$ Male and	Inhalation according to the	13 weeks (6 hours/day 5 days/	0, 50, 100, 200, 400, 800 ppm	Male and female: No dead animal Male: 50 ppm and above: suppression of body weight gain,	Senoh et al., 2003

Species sex/number of animals	Route	Period	Dose	Results	Reference
female 6 weeks 10 animals /group	OECD test guideline 413	week)	(0, 608, 1,216, 2,432 mg/m^3) mean actual concentra-tion: 0, 50.1, 100.3, 199.2, 400.2, 796.3 ppm (0, 152, 305, 606, 1,217, 2,421 mg/m^3)	increases in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and relative liver weight (absolute weight was also increased but not dose-dependently), centrilobular hepatocyte hypertrophy 50, 100 ppm: an increase in platelet count 100 ppm and above: focal hepatic necrosis (associated with ceroid and hemosiderin) 100, 400 ppm: an increase in total cholesterol 800 ppm: reduction in food consumption, increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities, massive hepatic necrosis (3 animals), hepatic single cell necrosis (associated with nucleolar plasmotomy) Female: 50 ppm and above: increases in MCV, MCH, total cholesterol, relative liver weight (dose-independently) 100 ppm and above: an increase in alkaline phosphatase (ALP) activity 200 ppm and above: an increase in ALT activity 800 ppm: an increase tendency in AST activity, increases in LDH activity and blood urea nitrogen (BUN), hepatic single cell necrosis (associated with nucleolar plasmotomy), centrilobular hepatocyte hypertrophy LOAEL : 50 ppm (in this assessment)	
Mouse ICR Male and female 55 days old 78 animals /group	Inhalation	18 months (6 hours/day 5 days/ week)	0, 25, 100, 400 ppm (0, 76, 304, 1,216 mg/m^3)	Male: 25 ppm and above: hepatocyte hypertrophy and single cell necrosis, lipofuscin and hemosiderin pigment in Kupffer cells 100 ppm: increases in absolute and relative liver weight 400 ppm: body weight gain (high value), enhanced body weight gain, increases in absolute and relative liver weight Female: 25 ppm and above: hepatocyte hypertrophy, single cell necrosis 100 ppm and above: enhanced body weight gain, lipofuscin and hemosiderin pigment in Kupffer cells 400 ppm: body weight gain (high value), enhanced body weight gain, increases in absolute and relative liver weight LOAEL: 25 ppm (76 mg/m^3) (in this assessment)	Malley et al., 1994
Mouse BDF_1 Male and female 6 weeks	Inhalation	104 weeks (6 hours/day 5 days/ week	0, 200, 400, 800 ppm (0, 608, 1,216, 2,432 mg/m^3)	Male: 200 ppm and above: dose-dependent suppression in body weight gain, increases in serum AST, ALT, γ-GTP, ALP and creatine phosphokinase (CPK) activities, total protein and cholesterol, increases	Senoh et al., 2004

Species sex/number of animals	Route	Period	Dose	Results	Reference
50 animals /group			mean actual concentra-tion: 0, 201.7, 397.8, 790.6 ppm (0, 613, 1,209, 2,403 mg/m^3)	in absolute and relative liver weight, red or brown hepatic node, centrilobular hepatocyte hypertrophy and nuclear atypiasingle cell necrosis, inflammatory cell foci 400 ppm and above: increases in serum albumin and BUN 800 ppm: an increase in serum total bilirubin Female: 200 ppm and above: dose-dependent suppression in body weight gain, red or brown hepatic nodes, increases in serum AST, ALT, γ-GTP, ALP and CPK activities, total bilirubin, protein, albumin and cholesterol, BUN and relative and absolute liver weight, centrilobular hepatocyte hypertrophy (excluding 400 ppm) 800 ppm: a decrease in survival rate (due to liver tumor), hepatocellular dyskaryosis See the section 7.3.7 for neoplastic and pre-neoplastic lesions.	
Rat SD Male young adult 10 animals /group	Inhalation	5 days (6 hours/ day)	0, 2,500 ppm (0, 7,600 mg/m^3)	2,500 ppm: 8/10 death Male: progressive infirmity, restlessness, body weight loss, dehydration, acute hepatic necrosis, acute pulmonary congestion, edema Surviving 2 animals were examined 10 days after the completion of exposure: recovered hepatic disorder was confirmed in one of them.	Kennedy & Sherman, 1986
Rat F344 Male and female 6 weeks 10 animals /group	Inhalation OECD according to the test guideline 412	2 weeks (6 hours/day 5 days/ week)	0, 100, 200, 400, 800, 1600 ppm (0, 304, 608, 1216, 2432, 4864 mg/m^3) mean actual concentra-tion: 0, 96.5, 197.6, 392.2, 779.1, 1554.4 ppm (0, 293, 601, 1,192, 2,368, 4,725 mg/m^3)	400 ppm and above: Female: suppression of body weight gain 800 ppm: Male and female: hepatic single cell necrosis associated with nucleolar plasmotomy 800 ppm and above: Male: suppression of body weight gain 1,600 ppm: death (3 males and 7 females), Male and female: an increase in relative liver weight, massive necrosis associated with hemorrhage, congestion, fibril formation and focal calcification of marked hepatic centrilobular cells in dead animals.	Senoh et al., 2003
Rat SD Female age unknown (approxi mately 200 g)	Inhalation	2 weeks (4 hours/day 5 days/ week)	0, 140 ppm (0, 420 mg/m^3)	140 ppm: lipidation around the hepatic lobules (10/11)	Lundberg et al., 1986
Rat	Inhalation	10-11 times (6 hours/day	0 ppm [1] 91 ppm	[1]-[3]: increase in the relative liver weight the results were similar between [1] and [2].	Clayton et al.,

Species sex/number of animals	Route	Period	Dose	Results	Reference
		5 days/ week)	10 days (6 hours/day, 5 days/week) [2] 1,104 ppm 10 days (0.5 hours/day, 5 days/week) [3] 91 ppm× 10 days (6 hours/day 5 days/week) 841 ppm 1 day (0.5 hours/day)	the condition of [3] was a combination of [1] and a single exposure for 0.5 hours at high concentration, and the relative liver weight of [3] was significantly higher than that of [1]. A short-term exposure of *N, N*-dimethylformamide at high concentration induces the same damage as that a long-term exposure at low concentration.	1963
Rat 3 to 12 weeks	Inhalation	28 days (8 hours/ day)	0, 200 ppm (0, 600 mg/m^3)	Increases in serum AST and ALT activities, morphological change in the liver (especially 3 weeks rat) No histopathological change in other organs	Tanaka, 1971
Rat Male and female age unknown 10 animals /group	Inhalation	58 times (6 hours/day 5 days/ week)	0, 23 ppm 5.5 hours 426 ppm 0.5 hours	No dead animal Male and female: increases in serum cholesterol, liver weight, slight increase in hepatic adipose	Clayton et al., 1963
Rat	Inhalation	9 weeks	0, 56 ppm 5 weeks(6 hours/day, 5 days/week,) 108 ppm× 4 weeks (6 hours/day, 5 days/week)	No effect	U.S. EPA, 1986
Rat F344 Male and female Young adult	Inhalation	12 weeks (6 hours/day 5 days/ week)	0, 150, 300, 600, 1,200 ppm mean actual concentra-tion: 0, 148.6, 302.4, 587.3, 1,184 ppm (0, 452, 919, 1,785, 3,599 mg/m^3)	Death: total 3 animals (300 ppm: 1 male, 1,200 ppm: 1 male and 1 female) Histopathological changes in dead animals: wide spread hepatolobular collaps, hepatic necrosis, accumulation of yellow-brown pigment in Kupffer cells, macrophages and hepatocytes, an increase in hepatic mitosis (one animal) Male: 150 ppm: clarified hepatic lobules (one animal) 600 ppm and above: slight decreases in hematocrit and hemoglobin 1,200 ppm: suppression of body weight gain, hepatlobular degeneration, yellow-brown pigment in Kupffer cells, macrophages and hepatocytes	Craig et al., 1984

Species sex/number of animals	Route	Period	Dose	Results	Reference
				Female: 150 ppm: hepatic discoloration (one animal) 300 ppm and above: hepatocyte anisokaryosis 600 ppm and above: an increase in alkaline phosphatase activity, yellow-brown pigment in Kupffer cells, macrophages and hepatocytes 1,200 ppm: suppression of body weight gain, clarified hepatic lobules (one animal), hepatomegaly (one animal), hepatlobular degeneration and fibril formation, large-sized hepatocytes LOAEL : 150 ppm (in this assessment)	
Rat F344 Male and female 51 day old 10 animals /group	Inhalation	13 weeks (6 hours/day 5 days/ week)	0, 50, 100, 200, 400, 800 ppm (0, 152, 304, 608, 1,216, 2,432 mg/m^3)	Male: 50 ppm and above: an increase in total cholesterol 100 ppm and above: a decrease in MCH, increases in platelet count and relative liver weight 200 ppm and above: decreases in MCV and alkaline phosphatase (ALP) activity, an increase in serum sorbitol dehydrogenase activity 400 ppm and above: suppression of body weight gain, increases in red blood cells (RBC), total bilirubin, ALT activities, a decrease in total protein, albumin (day 4: decrease, day 24 (400 ppm), day 91 (800 ppm): increase), centrilobular hepatic necrosis 800 ppm: increases in hematocrit, hemoglobin and isocitric dehydrogenase activity, macrophages containing pigment Female: 50 ppm and above: an increase in cholesterol, a decrease in total protein, an increase in relative liver weight 200 ppm and above: an increase in serum sorbitol dehydrogenase activity, a decrease in albumin 400 ppm and above: suppression of body weight gain, an increase in total bilirubin, centrilobular hepatic necrosis 800 ppm: increases in hematocrit, hemoglobin, RBC, ALT, isocitric dehydrogenase activity, and creatinine, a decrease in ALP activity, prolonged estrous cycle, macrophages containing pigment NOAEL: histopathological changes in the liver 200 ppm, changes in hepatic enzymes and an increase in liver weight was observed also in the lowest dose (determined by the authors) LOAEL: 50 ppm (in this assessment)	Lynch et al., 1991; Lynch et al., 2003 U.S. NTP, 1992
Rat F344 Male and female 6 weeks	Inhalation according to the OECD test guideline 413	13 weeks (6 hours/day 5 days/ week)	0, 50, 100, 200, 400, 800 ppm (0, 608, 1,216, 2,432 mg/m^3)	Male: 50 ppm and above: increases in total cholesterol and phospholipid 100 ppm and above: an increase in relative liver weight 200 ppm and above: hepatic single cell necrosis	Senoh et al., 2003

Species sex/number of animals	Route	Period	Dose	Results	Reference
10 animals /group			mean actual concentra-tion: 0, 49.6, 100.1, 199.5, 399.7, 795.6 ppm (0, 151, 304, 606, 1,215, 2,419 mg/m^3)	(sometimes associated with ceroid and hemosiderin, and nucleolar plasmotomy and cell division images) 400 ppm and above: suppression of body weight gain, centrilobular hepatocyte hypertrophy 800 ppm: reduction in feed consumption, increases in ALT, AST and LDH activities, a decrease in triglyceride, an increase in total bilirubin Female: 100 ppm and above: an increase in phospholipid 200 ppm and above: increases in totalt cholesterol, triglyceride and relative liver weight, hepatic single cell necrosis (sometimes associated with ceroid or hemosiderin, and nucleolar plasmotomy and cell division images) 400 ppm and above: suppression of body weight gain, increases in ALT and γ-GTP activities and total bilirubin, centrilobular hepatocyte hypertrophy 800 ppm: reduction in feed consumption, increases in AST and LDH activities and massive hepatolobular necrosis (one animal) LOAEL: 50 ppm (in this assessment)	
Rat SD Male and female 47 day old 87 animals /group	Inhalation	2 years (6 hours/day 5 days/ week)	0, 25, 100, 400 ppm (0, 76, 304, 1,216 mg/m^3)	Male: 25 ppm: No effect 100 ppm and above: suppression of body weight gain (body weight loss on exposure day 674 and later at 100 ppm), increases in sorbitol dehydrogenase activity and relative liver weight 400 ppm: centrilobular hepatocyte hypertrophy, single cell necrosis and centrilobular lipofuscin and hemosiderin pigment Female: 25 ppm: No effect 100 ppm and above: increases in SDH activity (only at 100 ppm) and relative liver weight, centrilobular hepatocyte hypertrophy 400 ppm: body weight loss (low value), suppression of body weight gain, an increase in sorbitol dehydrogenase activity, single cell necrosis, centrilobular lipofuscin and hemosiderin pigment NOAEL : 25 ppm (76 mg/m^3) (in this assessment)	Malley et al., 1994
Rat F344 Male and female 6 weeks 50 animals /group	Inhalation	104 weeks (6 hours/ day 5 days/ Week)	0, 200, 400, 800 ppm (0, 608, 1,216, 2,432 mg/m^3) mean actual concentra-tion: 0, 200.8, 399.9,	Male: 200 ppm and above: dose-dependent suppression in body weight gain, increases in AST, ALT, γ-GTP and ALP activities, total bilirubin and cholesterol, phospholipid, blood BUN and relative and absolute liver weight (excluding 400 ppm) 800 ppm: death (3 animals within 13 weeks), an increase in LDH activity, white or brown nodes in the liver of animals that survived for 2 years. Female:	Senoh et al., 2004

Species sex/number of animals	Route	Period	Dose	Results	Reference
			800.3 ppm (0, 610, 1,216, 2,433 mg/m^3)	200 ppm and above: dose-dependent suppression in body weight gain, increases in total bilirubin and cholesterol, phospholipid and relative and absolute liver weight (excluding 400 ppm) 400 ppm and above: an increase in ALP activity 800 ppm: death (13 animals within 21 weeks: due to hepatoc centrilobular necrosis), a decrease in food consumption, an increase in LDH activity, white or brown nodes in the liver of animals that survived for 2 years. See the section 7.3.7 for neoplastic and precancerous changes.	
Guinea pig Male age unknown 10 animals	Inhalation	58 times (6 hours/day 5 days/ week)	0, 23 ppm 5.5 hours 426 ppm 0.5 hour	No effects	Clayton et al., 1963
Rabbit	Inhalation	50 days (8 hours/ day)	0, 40 ppm (0, 120 mg/m^3)	Light- and electron-microscopic changes in the myocardium	Arena et al., 1982
Rabbit Male and female age unknown 2 animals /group	Inhalation	58 times (6 hours/day 5 days/ week)	0, 23 ppm 5.5 hours 426 ppm 0.5 hour	No dead animal Male: increases in serum cholesterol and liver weight	Clayton et al., 1963
Dog	Inhalation	3 weeks (6 hours/day 5 days/ week) (recovery: 4 weeks)	0, 50 ppm (0, 152 mg/m^3)	Changes in cardiac function (Rc). No effect on heart sound.	U.S. EPA, 1986
Dog	Inhalation	28 days (6 hours/ day)	0, 21 ppm (0, 63 mg/m^3)	No effects on plasma ALT, AST, bilirubin, BUN and creatinine	Kimmerle & Eben, 1975a
Dog Male age unknown 4 animals	Inhalation	58 times (6 hours/day 5 days/ week)	0, 23 ppm 5.5 hours 426 ppm 0.5 hour	No dead animal Male: slight decreases in heart rate velocity and blood pressure sound, a decrease in systolic pressure, increases in RBC count and concentration, slight polyuria, increases in plasma cholesterol, alkaline and phosphatase, cholinesterase, bromsulfalein retention, myocardial changes Histological effect: found in the liver, pancreas, spleen, adrenal grand and thymus (details unknown)	Clayton et al., 1963
Cynomolgus monkey	Inhalation	2 weeks (6 hours/day 5 days/ week)	0, 500 ppm (0, 1,520 mg/m^3)	No effects	Hurtt et al., 1991
Cynomolgus	Inhalation	13 weeks (6 hours/day	0, 30, 100, 500 ppm	No effects	Hurtt et al., 1991

Species sex/number of animals	Route	Period	Dose	Results	Reference
monkey 3 animals Male and female age unknown		5 days/ week) (recovery: 90 days)	(0, 91, 304, 1,520 mg/m^3)		
Rat	Dermal:	28 days	[1] 0, 960 mg/kg/day [2] 1,920 mg/kg/2 days [3] 1,920 mg/kg/day 2 days + 0 mg/kg/day× 2 days	Hepatic function, biochemical and pathological changes, lipid metabolism change (details unknown)	Bainova et al., 1981
Rat	Dermal	30 days	0, 215, 430, 960, 4,800 mg/kg/day	215 mg/kg: No effect 430 mg/kg and above: dose-dependent ATP, AST, ALP, cholinesterase, and γ-GTP changes NOAEL : 215 mg/kg (evaluated by IPCS)[1)]	Bainova & Antov, 1980
Rabbit	Dermal	7 days 2 mL/dose 3 times /day	0, 50, 100% aqueous solution	100%: death 5-8 days after the completion of administration, biochemical and histopathological changes in the liver (details unkown)	Huang et al., 1981
Rabbit NZW 6 group (No descrip-tion of sex)	Dermal (shaven hair)	2 weeks (9 times) recovery: 4, 11 days	0, 2,000 mg/kg	2,000 mg/kg: 4 dead animals body weight loss, anorexia, infirmity and cyanosis, and hepatic congestion and necrosis	Kennedy & Sherman, 1986
Guinea pig	Dermal	7 days (2 mL/dose 3 times /day)	0, 50, 75, 100% aqueous solution	50%: died 4-9 days after the completion of administration 75, 100%: died 2-4 days after the completion of administration body weight loss, hepatic damage	Huang et al., 1981
Rat Wistar Male	Subcutan-eous	1 week	0, 474.5 mg (0.5 mL) /kg/day	increases in ATP, AST and cholinesterase activities, increases in total chlesterol, and decreases in hepatic P450 and glutathione reductase activity	Imazu et al., 1992

7.3.5 Reproductive and developmental toxicity

Studies on the reproductive and developmental toxicity of *N, N*-dimethylformamide to experimental animals are summarized in Table 7-6.

An oral administration (via drinking water) study of *N, N*-dimethylformamide in male and female ICR mice (F_0) at concentrations of 0, 1,000, 4,000 and 7,000 ppm (0, 200 to 1,300 mg/kg/day) for 14 weeks was conducted. In F_0 mice, an increase in liver weight at 1,000 ppm and above, reduction in fertility at 4,000 ppm, and body weight loss at 7,000 ppm were observed. In F_2 mice from F_1 given at 1,000 ppm and above,

decreases in the number of litters, body weight of surviving fetuses, anomaly of the cranial and sternal bones were found, and in F_2 mice from F_1 given at 4,000 ppm and above, a decrease in survival rate and body weight loss were observed (Fail et al., 1998).

In an oral administration (gavage) study of *N, N*-dimethylformamide at doses of 0, 182 and 548 mg/kg/day in NMRI mice from gestation day 6 to 15, fetal body weight loss, increases in delayed development and variations, and anomaly (palate cleft, exencephalia, hydrocephalia, sphenoid defect and rib synostosis) were observed at 182 mg/kg/day and above (Hellwig et al., 1991).

In an intraperitoneal administration study of *N, N*-dimethylformamide at doses of 0, 378 and 944 mg/kg/day in NMRI mice from gestation day 11 to 15, body weight loss and suppression of body weight gain, death (hepatic necrosis, fatty liver), stillbirth, and a decrease in the number of litters at a dose of 944 mg/kg/day were observed in F_0, and exencephalia, palate cleft and an increase in resorption in F_1 (Hellwig et al., 1991). In a similar intraperitoneal administration study of *N, N*-dimethylformamide at doses of 0, 170, 250, 600 and 1,100 mg/kg/day in mice from gestation day 1 to 14, F_1 developed defect or delay of occipital bone formation at doses of 600 mg/kg/day and above, open eyelids, cerebral edema, sternal hematoma, and bifid thoracic vertebra (Scheufler and Freye, 1975).

In an oral administration (gavage) study of *N, N*-dimethylformamide at doses of 0, 50, 100, 200 and 300 mg/kg/day in SD rats from gestation day 6 to 20, suppression of body weight gain and reduction in feed consumption were observed in F_0 rats at doses of 100 mg/kg/day and above, and fetal body weight loss at doses of 100 mg/kg/day and above, and skeletal variations in supraoccipital and sternebrae at doses of 200 mg/kg/day and above in F_1 rats. The NOAEL was reported as 50 mg/kg/day (Saillenfait et al.,1997). In a similar oral administration (gavage) study at doses of 166, 503 and 1,510 mg/kg/day from gestation day 6 to 15, an increase in resorption, a decrease in placenta weight, tail defect, systemic edema, micrognathia, and abnormal rib, sterna and spine were observed at 1,510 mg/kg/day (Hellwig et al., 1991).

In an inhalation exposure study of *N, N*-dimethylformamide in SD rats at concentrations of 0, 32 and 301 ppm (corresponding to 0, 97 and 915 mg/m^3) from gestation day 6 to 15, F_0 rats showed body weight loss during exposure period at 301 ppm, and fetal body weight loss and an increase in the incidence of variation of ossifications were observed in F_1 rats (Keller and Lewis, 1981). The NOAEL of this study is considered to be as 32 ppm in this assessment. In a similar inhalation exposure study at a concentration of 0, 287 ppm (corresponding to 0, 872 mg/m^3) for 6 hours/day in several gestation days, F_0 rats showed suppression of body weight gain, increases in resorption and dead fetuses, fetal body weight loss and an increase in abnormal sternal bone and growth-retardation(Hellwig et al., 1991). In an inhalation exposure study at concentrations of 0, 18 and 172 ppm (0, 55 and 523 mg/m^3) from gestation day 6 to 15, body weight loss was observed at 172 ppm (Kimmerle and Machemer, 1975a), and in a inhalation exposure study at 400 ppm (1,216 mg/m^3) for 4 hours/day from gestation day 10 to 20, fetal death was increased (Schottek, 1964). However, the details are unknown.

In an single intravenous administration study of *N, N*-dimethylformamide in Wistar rats at a single dose of 0, 45 mg/kg either on gestation day 10, 11 or 12, in F_1 rats from the parents administered *N, N*-dimethylformamide on gestation day 11, rib malformation and a decrease in fetal body weight were observed. F_1 ratsfrom the parents administered *N, N*-dimethylformamide on gestation day 12, developed

malformation in the spine and eyes (eyeball rupture and microphthalmia) (Parkhie and Webb, 1983).

In a dermal application study of *N, N*-dimethylformamide in SD rats at a dose of 2 mL/kg/day from gestation day 6 to 15 or 1 to 20, F_0 rats showed body weight loss, supression of body weight gain and a decrease in pregnancy rate, and F_1 rats, an increase in postimplantation embryo lethality and decreases in surviving fetuses and fetal body weight (Hansen and Meyer, 1990). In a similar dermal administration study at doses of 0, 94, 472 and 944 mL/kg/day from gestation day 6 to 10 or 13 to 15, F_0 rats showed body weight loss and dermatitis at 944 mL/kg/day (Hellwig et al., 1991).

In a dermal administration study of *N, N*-dimethylformamide in rats at doses of 0, 600, 1,200 and 2,400 mg/kg from gestation day 9 to 13, F_0 rats showed suppression of body weight gain at doses of 600 and 1,200 mg/kg/day, and F_1 rats showed an increase in mortality at doses of 600 to 2,400 mg/kg/day (Stula and Krauss, 1977).

In an oral administration (gavage) study of *N, N*-dimethylformamide in rabbits at doses of 0, 46.4, 68.1 and 200 μL/kg/day from gestation day 6 to 18, F_0 rabbits showed a reduction in food consumption and suppression of body weight gain at 200 μL/kg/day, and F_1 rabbits developed hydrocephalus at doses of 46.4 and 68.1 μL/kg/day, reduction in implantation rate at a dose of 68.1 μL/kg/day, and body weight loss, a decrease in placenta weight, omphalocele, eventration, exophthalmus, palate cleft, and abnormal extremity position at 200 μL/kg/day (Merkle and Zeller, 1980).

An inhalation exposure study of *N, N*-dimethylformamide in Himalayan rabbits at concentrations of 0, 50, 150 and 450 ppm (corresponding to 0, 152, 456 and 1,368 mg/m^3) for 6 hours/day from gestation day 7 to 19 was carried out. In F_0 rabbits, abortion was found at 150 ppm, and suppression of body weight gain at 450 ppm, and in F_1 rabbits at 450 ppm, anomalies of amniocele, bladder defect, abnormal sternal bone and spina bifida were observed (Hellwig et al., 1991).

In a dermal admistration study of *N, N*-dimethylformamide in Himalayan rabbits at doses of 100, 200 and 400 mg/kg/day from gestation day 6 to 18, at 400 mg/kg/day, F_0 rabbits developed dermatitis, and F_1 rabbits showed amniocele and bladder defect (Hellwig et al., 1991).

In a dermal study in rabbits at a dose of 200 mg/kg/day from gestation day 8 to 16, F_1 rabbits showed increases in mortality and body weight (Stula and Krauss, 1977).

In many studies, *N, N*-dimethylformamide showed reproductive and developmental toxicty, and the NOAEL of *N, N*-dimethylformamide for oral administration is determined as 50 mg/kg/day based on the results of the teratogenicity study in rats (Saillenfait et al., 1997), and the NOAEL for inhalation exposure is determined as 32 ppm (corresponding to 97 mg/m^3) based on the results of the teratogenicity study in rats (Keller and Lewis, 1981).

Table 7-6 Reproductive and developmental toxicity of *N, N*-dimethylformamide

Species sex/numbers of animals	Route	Period	Dose	Results	Reference
Mouse ICR Male and female	Drinking water	14 weeks	0, 1,000, 4,000, 7,000 ppm (corresponding to 0, 200, 800, 1,300 mg/kg/day)	$F_{0:}$ 1,000 ppm and above: Male and female: increase in liver weight 4,000 ppm: reduction in fertility 7,000 ppm: Female: body weight loss $F_{1:}$ 1,000 ppm and above: decreases in the number of litters, body weight of surviving fetuses (F_2) anomalies of the cranial and sternal bones in adults 4,000 ppm and above: Male and female: a decrease in postnatal survival rate and body weight loss	Fail et al., 1998
Mouse NMRI 26/group	Oral gavage	Gestation day 6-15	0, 182, 548 mg/kg/day	$F_{0:}$ 182 mg/kg/day and above: no effect $F_{1:}$ 182 mg/kg/day and above: fetal body weight loss, increases in delayed development, variation and anomaly (palate cleft, exencephalia, hydrocephalus, sphenoid defect and rib synostosis)	Hellwig et al., 1991
Mouse NMRI	Intraperi-toneal	Gestation day 11-15	0, 378, 944 mg/kg/day	$F_{0:}$ 944 mg/kg/day: body weight loss 2/8 death (hepatic necrosis and fatty liver) stillbirth in 2 animals a decrease in the number of litters (6 animals) $F_{1:}$ 944 mg/kg/day: exencephalia in 7/36 animals, palate cleft in 1/36 animals, an increase in resorption,	Hellwig et al., 1991
Mouse	Intraperi-toneal	Gestation day 1-14 (170, 1,100 mg/kg) Gestation day 6-14 (250 mg/kg)	0, 170, 250, 600, 1,100 mg/kg/day	$F_{0:}$ 170 mg/kg/day and above: no effects $F_{1:}$ 600 mg/kg/day and above: resorption delay, anomaly (defect or delay of occipital bone formation (600 mg/kg/day: 18%, 1,100 mg/kg/day: 75%), open eyelids, cerebral edema, sternal hematoma, and bifid thoracic vertebra)	Scheufler & Freye, 1975

Species sex/numbers of animals	Route	Period	Dose	Results	Reference
Rat SD	Oral gavage	Gestation day 6-20	0, 50, 100, 200, 300 mg/kg/day	$F_{0:}$ 100 mg/kg/day and above: Suppression of body weight gain and reduction in food consumption $F_{1:}$ 100 mg/kg/day and above: Fetal body weight loss 200 mg/kg/day and above: skeletal variations in supraoccipital and sternebrae NOAEL : 50 mg/kg/day	Saillenfait et al., 1997
Rat SD	Oral gavage	Gestation day 6-15	0, 166, 503, 1,510 mg/kg/day	$F_{0:}$ no effect $F_{1:}$ 1,510 mg/kg/day: An increase in resorption, a decrease in placenta weight, tail defect, systemic edema, micrognathia, and abnormal rib, sterna and spine	Hellwig et al., 1991
Rat SD	Inhalation	Gestation day 6-15 6 hours /day	0, 32, 301 ppm (0, 97, 915 mg/m^3)	F_0: 301 ppm: body weight loss $F_{1:}$ 301 ppm: fetal body weight loss, an increase in the incidence of variation in ossification NOAEL : 32 ppm (in this assessment)	Keller & Lewis, 1981
Rat SD	Inhalation	Experi-ment I (Gestation day 0-1, 4-8, 11-15, 18-19) Experi-ment II (Gestation day 0-3, 6-10, 11-18)	0, 287 ppm, (0, 872 mg/m^3) 6 hours/day	$F_{0:}$ 287 ppm: suppression of body weight gain increases in resorption and dead fetuses fetal body weight loss and an increase in abnormal sternal bone and growth-retardation	Hellwig et al., 1991
Rat	Inhalation	Gestation day 6-15	0, 18, 172 ppm (0, 55, 523 mg/m^3)	F_1: 172 ppm: body weight loss	Kimmerle & Machemer, 1975
Rat	Inhalation	Gestation day 10-20	0, 400 ppm (0, 1,216 mg/m^3) 4 hours/day	F_1: An increase in dead fetuses	Schottek, 1964

Species sex/numbers of animals	Route	Period	Dose	Results	Reference
Rat	Inhalation	Gestation day 0-20	0, 16, 200 ppm (0, 49, 1848 mg/m^3)	F_0: no effects F_1: 16 ppm: body weight loss 200 ppm: An increase in mortality, body weight loss	Sheveleva & Osina, 1973
Rat Wistar	Intraven-ous	Once on gestation day 10, 11 or 12	0, 45 mg/kg (corresponding to 0, 90 mg/mL)	F_1: Administration on gestation day 11 Rib anomaly Fetal body weight loss Administration on gestation day 12 Spine anomaly Eye anomaly (eyeball rupture and microphthalmia)	Parkhie and Webb, 1983
Rat SD	Dermal	Gestation day 6-15 or Gestation day 1-20	0, 2 mL/kg	F_0: body weight loss reduction in body weight gain and pregnancy rate F_1: an increase in postimplantation embryo lethality and decreases in surviving fetuses	Hansen and Meyer, 1990
Rat SD	Dermal	Gestation day 6-10, 13-15	0, 94, 472, 944 mg/kg/day	F_0: 944 mg/kg/day: body weight loss and dermatitis	Hellwig et al., 1991
Rat	Dermal	Gestation day 9-13	0, 600, 1,200, 2,400 mg/kg/day	F_0: 600 mg/kg/day and above: suppression of body weight gain F_1: 600 mg/kg/day and above: An increase in mortality	Stula & Krauss, 1977
Rabbit	Oral administ-ration	Gestation day 6-18	0, 46.4, 68.1, 200 µL/kg/day	F_0: 200 µL/kg/day: Reduction in feed consumption, suppression of body weight gain, a decrease in placenta weight F_1: 46.4-68.1 µL/kg/day: hydrocephalus 68.1 µL/kg/day: reduction in implantation rate 200 µL/kg/day: body weight loss, a decrease in placenta weight, amniocele, eventration, exophthalmus, palate cleft, and abnormal extremity position	Merkle & Zeller, 1980

Species sex/numbers of animals	Route	Period	Dose	Results	Reference
Himalayan rabbit	Inhalation	Gestation day 7-19	Control group with air, 0, 50, 150, 450 ppm (0, 152, 456, 1,368 mg/m^3) 6 hours/day	F_0: 150 ppm: abortion 450 ppm: suppression of body weight gain F_1: 450 ppm: anomaly (amniocele, bladder defect, abnormal sternal bone, and spondyloschisis)	Hellwig et al., 1991
Himalayan rabbit	Dermal	Gestation day 6-18	0, 100, 200, 400 mg/kg/day	F_0: 400 mg/kg/day: dermatitis F_1: 400 mg/kg/day: amniocele and bladder defect	Hellwig et al., 1991
Rabbit	Dermal	Gestation day 8-16	0, 200 mg/kg/day	F_1: increases in mortality and body weight	Stula & Krauss, 1977
Cynomolgus monkey	Inhalation	13 weeks	0, 30, 100, 500 ppm (0, 91.2, 304, 1,520 mg/m^3)	30 ppm and above: No effects (semen volume, sperm count, percentage of motile sperms and sperm morphology)	Hurtt et al., 1991

7.3.6 Genotoxicity

Studies on the genotoxicity of *N, N*-dimethylformamide are summarized in Table 7-7.

a. *in vitro* studies

Many studies have been conducted to assess genotoxicity of *N, N*-dimethylformamide. Many reverse mutation assays *in Salmonella typhimurium* (Antoine et al., 1983; Brams et al., 1987; E.I. DuPont de Nemours, 1976; Mortelmans et al., 1986; Richold and Jones, 1981) and DNA repair assay (Serres and Ashby, 1981) revealed negative results, and of studies with bacteria, only one reverse mutation assay (Trueman, 1981) and an mitotic recombination assay with yeast (Serres and Ashby, 1981) reported positive results. Also, no genotoxicity was found in chromosomal aberration test with yeast (Serres and Ashby, 1981) and in mitotic mutation assay (Serres and Ashby, 1981).

The results were negative in many mutation tests (Jotz and Mitchell, 1981; Mitchell et al., 1988; Myhr and Caspary, 1988), chromosomal aberration test in Chinese hamster ovary (CHO) cells, human peripheral lymphocytes (Antoine et al., 1983; Natarajan and Van Kesteren-van Leeuwen, 1981), sister chromatid exchange tests (Antoine et al., 1983; Evans and Mitchell, 1981; Natarajan and Van Kesteren-van Leeuwen, 1981; Parry and Thomson, 1981; Serres and Ashby, 1981) and unscheduled DNA synthesis assays (Martin and McDermid, 1981; Serres and Ashby, 1981). In the studies with cultured cells, a mutation test with mouse lymphoma cells (McGregor et al., 1988) and a chromosomal aberration test with human lymphocytes (Koudela and Spazier, 1979) reported positive results only at high doses. In a cell transformation assay with hamster BHK21 cells (Serres and Ashby, 1981), positive and negative results were obtained.

b. *in vivo* studies

In *in vivo* studies, chromosomal aberration (Sheveleva et al., 1979), dominant lethal tests in rats (Lewis et al., 1979) and micronucleus tests in mice (Antoine et al., 1983; Kirkhart, 1981; Salamone et al., 1981; Serres and Ashby, 1981; Tsuchimoto and Matter, 1981) showed negative results.

As summarized above, negative results were obtained in the majority of *in vitro* genotoxicity studies with *N, N*-dimethylformamide, and also the available *in vivo* studies showed negative results. The overall evaluation of these data indicates that *N, N*-dimethylformamide is not genotoxic.

Table 7-7 Genotoxicity of *N, N*-dimethylformamide

	Test system	Species (Organisms) /Strain	Experimental condition	Concentration / Dose	Results [a), b)] - S9 +S9	Reference
in vitro	Reverse mutation	*Salmonella typhimurium* TA1535, TA1537, TA100, TA1538 ,TA98	Plate incorporation method	2,000-10,000 μg/mL	- -	E.I. DuPont de Nemours, 1976
		Salmonella typhimurium TA97, TA98,TA100	Plate incorporation method	0, 50,000-200,000 μg/mL	- -	Brams et al., 1987
		Salmonella typhimurium TA1535, TA98, TA100, TA1537	Preincubation method rat and hamster S9	100 - 10,000 μg/plate	- -	Mortelmans et al., 1986
		Salmonella typhimurium TA1535, TA98, TA100, TA1538, TA1537	Plate incorporation method	0.65×10^{-5} - 1.3×10^{-3}M	- -	Antoine, et al., 1983
		Salmonella typhimurium TA1535, TA98 TA100, TA1538, TA1537	ND	10-10,000 μg/mL	- -	Richold & Jones, 1981
		Salmonella typhimurium TA1535, TA98, TA100, TA1538, TA1537	ND	4-2,500 μg/mL	- + (600) - + (Unknown) -	Trueman, 1981
		Salmonella typhimurium TA100, TA98	without S9 mix incubation method 16 to 18 hours at 37°C	0, 1-500 mg/mL	- -	Habbad et al., 1981
	SOS repair	*Escherichia coli* PQ 37	ND	0, 7.3 ng/mL - 7.3 mg/mL	- -	Brams et al., 1987
	rec assay	*Bacillus subtilis*	Rat S9	20 mg/disk	- -	Serres & Ashby, 1981
		Escherichia coli 2921、9239、8471、5519、7623、7689	ND	1 g/mL	-	Serres & Ashby, 1981
	DNA repair	*Escherichia coli* W3110、P3478	without S9 mix	100 μL/mL	-	Serres & Ashby, 1981
	Mitotic re-combinetion	*Saccharomyces cerevisiae* JD1	ND	ND	+	Serres & Ashby, 1981

	Test system	Species (Organisms) /Strain	Experimental condition	Concentration / Dose	Results [a), b)] - S9 +S9	Reference
	Mitotic crossing-over assay	*S.cerevisiae* T1, T2	ND	10-1,000 µg/mL	-	Serres & Ashby, 1981
	Chromosome aberration (Aneuploid)	*S.cerevisiae* D6	ND	100 µg/mL	- ND	Serres & Ashby, 1981
	Gene conversion	*S.cerevisiae* D7	ND	5 µL/mL	ND -	Serres & Ashby, 1981
	DNA repair test in yeasts (cell growth inhibition)	*S.cerevisiae* wild & rad	ND	300 µg/mL	+	Serres & Ashby, 1981
	Chromosome aberration	CHO cells	1 hour treatment	1.67 - 6.67 µL/mL	- -	Natarajan & Van Kesteren-van Leeuwen, 1981
		Human peripheral lymphocytes	24 hours treatment	1.1×10^{-2} - 1.1 M	-	Antoine et al., 1983
		Human peripheral lymphocytes	ND	10-20%	+	Koudela & Spazier, 1979
	Mouse lymphoma (TK locus)	Mouse lymphoma L5178Ycells	Aroclor 1254-induced S9	0, 125 - 5,000 nL/mL	- -	Myhr & Caspary, 1988
		Mouse lymphoma L5178Y cells	37°C 4 hours treatment	46.9 - 3,000 µg/mL	- -	Jotz & Mitchell, 1981
		Mouse lymphoma L5178Y cells	4 hours treatment	1.3 – 5 µL/mL	- -	Mitchell et al., 1988a, b
		Mouse lymphoma L5178Y cells	4 hours treatment	312.5 - 5,000 µg/mL	+ - (5,000)	McGregor et al., 1988
	Sister chromatid exchange	CHO cells	-S9; 37°C, 21.5h +S9; 37°C, 2 hours, 21.5 hours recovery	0.00625-0.1 %	- -	Evans & Mitchell, 1981
		CHO cells	1 hour treatment	1.67-6.67 µL/mL	- -	Natarajan & Van Kesteren-van Leeuwen, 1981
		CHO cells	1 hour treatment	0.01-10 µg/mL	- -	Parry & Thomson, 1981
			24 hours treatment	10 µg/mL	- ND	
		Human peripheral lymphocytes cells	24 hours treatment	1.1×10^{-2}-1.1 M	-	Antoine, et al., 1983
		CHO cells	ND	+S9; 0.00625-0.1% -S9; 0.1-100 µg/mL	-	Serres & Ashby, 1981

	Test system	Species (Organisms) /Strain	Experimental condition	Concentration / Dose	Results [a), b)] - S9 +S9	Reference
	DNA repair	$B6C3F_1$ mouse and Syrian hamster primary hepatocytes	Hepatocytes were simultaneously exposed to the test chemical and ^3H-thymidine for 18 hours.	10^{-2} M	- -	McQeen et al., 1983
	Inhibition of metabolic cooperation	Chinese hamster V79 cells, wild type (6TGS, HGPRT+), mutant (6TGr , HGPRT)	ND	20-45 µL/5mL	+ (20 - 45µL/5mL)	Chen et al., 1984
	Unscheduled DNA synthesis	HeLa S3 cells	S9 (Phenobarbitone- and 3-methylcholanthrene- induced liver of Wistar rat)	0.1-100 µg/mL	- -	Martin & McDermid, 1981
		Human fibroblast WI-38 cells	ND	1.1-90µg/mL (-S9) 2-30 mg/mL (+S9)	- -	Serres & Ashby, 1981
		Human fibroblast cells (skin biopsies)	ND	0.032-100 µg/mL	-	
		HeLa cells	ND	0.1-100 µg/mL	-	
	Mutation (diphteria toxin resistance)	Human lung fibroblast cells (HSC172)	ND	0.2-0.5 mg/mL	-	Serres & Ashby, 1981
	Trans-formation	Liver cells of newborn hamster (BHK21C13 /HRC1) BHK21	ND	500 µg/mL	+ -	Serres & Ashby, 1981
in vivo	Chromosome aberration test	female and male rat	Inhalation exposure	0.77-201 ppm	-	Sheveleva et al., 1979
	Micro-nucleus	ICR mouse male	Femoral marrow smears were made using four animals per group at 30 hours and four at 48 hours after intraperitoneal injection.	0, 0.425, 0.85, 1.70 mg/kg	-	Kirkhart, 1981
		ICR mouse	intraperitoneal injection	0.425-1.7 mg/kg	-	Serres & Ashby, 1981
		BALB/c mouse male	Single, intraperitoneal injection	0.2 - 2,000 mg/kg	-	Antoine, 1983
		Hybrid mouse, $B6C3F_1$, for each 5	Phase 1: twice, every 24 hours Phase 2: single	Phase 1: 80% LD_{50}/7 Phase 2: 80, 50% LD50/7 LD50/7 (the dose required to kill 50% of the animals within 7 days)	- -	Salamone et al., 1981
		$B6C3F_1$mouse	Intraperitoneal injection	80% of LD_{50}	-	Serres & Ashby, 1981

	Test system	Species (Organisms) /Strain	Experimental condition	Concentration / Dose	Results [a), b)] - S9 +S9	Reference
		ICR mouse female and male	Twice, every 24 hours, intraperitoneal injection, for each 2	0.4-1.6 mg/kg	-	Tsuchimoto & Matter, 1981
		ICR mouse	Intraperitoneal injection	0.4-1.6 mg/kg	-	Serres & Ashby, 1981
	Sex-linked recessive lethal	*Drosophila melanogaster*, Berlin K (wild type), Basc, In (1) $sc^{s1L}sc^{8R}$+S, $sc^{s1}sc^{8}w^{a}B$	Berlin K (wild type) males were fed for 3 days.	0.2 % (v/v)	-	Wurgler & Graf, 1981
	Sperm abnormality	BALB/c male mouse	Single intraperitoneal injection	0.2-2,000 mg/kg	-	Antoine, 1983
		(CBA×BALB/c) F_1 male mouse	Intraperitoneal injection	0.1-1.5 mg/kg	-	Serres & Ashby, 1981
	Dominant lethal	Male rat	5 days 6 hours/day	30.1-301 ppm	-	Lewis et al., 1979

a) -: Negative +: Positive ND: No data available

b) Positive reaction doses are in parentheses. (μg/plate)

7.3.7 Carcinogenicity

Studies on the carcinogenicity of *N, N*-dimethylformamide are summarized in Table 7-8.

Regarding carcinogenicity of *N, N*-dimethylformamide, an inhalation exposure study in ICR mice and SD rats (Malley et al., 1994) and an oral (via drinking water) and subcutaneous administration study in BD rats (Druckrey et al., 1967) were conducted. The latter study was conducted with a small number of animals and the results reported were insufficient, which therefore, could not be used for carcinogenicity evaluation.

In an inhalation exposure study of *N, N*-dimethylformamide in male and female ICR mice at concentrations of 0, 25, 100 and 400 ppm for 18 months, effects on the liver were observed at 25 ppm and above, however, no carcinogenicity was found (Malley et al., 1994).

In an inhalation exposure study of *N, N*-dimethylformamide in BDF_1 mice at concentrations of 0, 200, 400 and 800 ppm for 104 weeks (6 hours/day, 5 days/week), development of neoplastic lesions, i.e., hepatocellular adenoma and carcinoma and hepatic blastoma were increased in males and females at 200 ppm (corresponding to 152 mg/m^3) and above. In males and females at 200 ppm and above, small foci of altered hepatocytes (clear and eosinophilic cell foci), prenoeplastic changes were observed (Senoh et al., 2004).

In a 2-year inhalation exposure study of *N, N*-dimethylformamide in male and female SD rats at concentrations of 0, 25, 100 and 400 ppm, effects on the liver were observed at 100 ppm and above, and increases in clear cell foci in male and female and eosinophilic foci of cellular alteration in female were found at 400 ppm, but no tumor developed (Malley et al., 1994).

In a inhalation exposure study of *N, N*-dimethylformamide in F344 rats at concentrations of 0, 200, 400 and 800 ppm for 104 weeks (6 hours/day, 5 days/week), hepatocellular adenoma was observed in males and

females at 400 ppm (corresponding to 1,216 mg/m^3) and above, and hepatocellular carcinoma at 800 ppm (corresponding to 2,432 mg/m^3), and the incidences of hepatocellular adenoma and carcinoma were increased in males and females. The increased incidences of hepatocellular adenoma and carcinoma showed dose-dependent trends. As preneoplastic changes, clear cell foci were observed in males at 400 ppm and above and females at 200 ppm and above, and eosinophilic cell foci in males and females at 400 ppm and above groups, and mixed cell and vacuolated cell foci in males at 800 ppm (Senoh et al., 2004).

Senoh et al. (2004) compared the results with those of Malley et al. (1994) and considered that the differences in tumor development in the mice and rats exposed at 400 ppm were attributable to the strain differences, and furthermore, the difference in tumor development between ICR and BDF_1 mice was caused by the difference in the administration period (ICR: 1.5 years and BDF_1: 2 years). In addition, Senoh et al. indicated that sensitivity to hepatic tumor was higher in mice than that in rats.

Based on the results that hepatic tumors were developed in inhalation exposure studies in BDF_1 mice and F344 rats (Senoh et al., 2004), *N, N*-dimethylformamide is considered carcinogenic to experimental animals in inhalation exposure.

The evaluations of carcinogenicity of *N, N*-dimethylformamide by the international and national organizations are shown in Table 7-9. The IARC has categorized *N, N*-dimethylformamide as Group 3 (not classifiable as to its carcinogenicity to humans).

Table 7-8 Carcinogenicity of *N, N*-dimethylformamide

Species	Route	Period	Dose	Results	Reference
Mouse ICR Male and female 55 day old 78 animals /group	Inhala-tion	18 months (6 hours/day 5 days/ week)	0, 25, 100, 400 ppm (0, 76, 304 1,216 mg/m^3)	Carcinogenicity was not confirmed under the conditions in this experiment.	Malley et al., 1994
Mouse BDF_1 Male and female 6 weeks 50 animals /group	Inhala-tion	104 weeks (6 hours/day 5 days/ week)	0, 200, 400, 800 ppm (0, 608, 1,216, 2,432 mg/m^3) mean actual concentration: 0, 201.7, 397.8, 790.6 ppm (0, 613, 1,209, 2,403 mg/m^3)	Histopathological change (liver) [1)] Group (ppm) 0 200 400 800 Neoplastic lesion Male Hepatocellular adenoma# 6 36** 41** 41** Hepatocellular carcinoma# 2 12** 16** 16** Hepatic blastoma 0 13** 7** 4 Total of tumor# [2)] 8 42** 46** 44** Female Hepatocellular adenoma# 1 42** 47** 48** Hepatocellular carcinoma# 3 25** 32** 35**	Senoh et al., 2004

Species	Route	Period	Dose	Results	Reference
				Hepatic blastoma 0 0 4 0 Total of tumor# [2] 3 45** 49** 49** <u>Altered hepatocyte</u> Male Clear cell foci 4 21** 13** 17** Eosinophilic cell foci 1 38** 41** 42** Female Clear cell foci 3 7 4 2 Eosinophilic cell foci 1 43** 43** 48** #: Significant by Peto test (P<0.01) * : Significant by Fisher exact test (P<0.05) **: Significant by Fisher exact test (P<0.01) 1) : No effect of *N, N*-dimethylformamide on organs other than the liver 2) : Hepatocellular adenoma, carcinoma or hepatic blatoma Nonneoplastic changes other than altered hepatocyte are described in the repeated toxicity (Section 7.3.3).	
Rat SD Male and female 47 day old 87 animals /group	Inhala-tion	2 years (6 hours/day 5 days/ week)	0, 25, 100, 400 ppm (0, 76, 304 1,216 mg/m^3)	Histopathological change (liver) <u>Group (ppm) 0 25 100 400</u> <u>Altered hepatocyte</u> Male Clear cell foci 11 8 22* 35* Eosinophilic cell foci 33 36 24 45 Female Clear cell foci 5 5 14 24* <u>Eosinophilic cell foci 22 12 25 40*</u> * : Significant by Fisher exact test (P<0.05) Carcinogenicity was not confirmed in this condition.	Malley et al., 1994
Rat F344 Male and female 6 weeks 50 animals /group	Inhala-tion	104 weeks (6 hours/day 5 days/ Week)	0, 200, 400, 800 ppm (0, 608, 1,216, 2,432 mg/m^3) mean actual concentration: 0, 200.8, 399.9, 800.3 ppm (0, 610, 1,216, 2,433 mg/m^3)	Histopathological change (liver) [1] <u>Group (ppm) 0 200 400 800</u> <u>Neoplastic lesion</u> Male Hepatocellular adenoma# 1 3 13** 20** Hepatocellular carcinoma# 0 1 0 24** Total of tumor[2] # 1 4 13** 33** Female Hepatocellular adenoma# 1 1 6 16** Hepatocellular carcinoma# 0 0 0 5* Total of tumor#[2]	Senoh et al., 2004

Species	Route	Period	Dose	Results	Reference
				1 1 6 19** Altered hepatocyte Male Clear cell foci 11 21 35** 40** Eosinophilic cell foci 13 14 34** 40** Basophil cell foci 24 26 29 42** Mixed cell foci 0 0 1 6* Vacuolated cell foci 6 0* 7 16* Female Clear cell foci 3 23** 33** 33** Eosinophilic cell foci 0 4 10** 20** Basophil cell foci 23 27 15 29 Mixed cell foci 0 0 0 1 Vacuolated cell foci 0 0 1 3 #: Significant by Peto test (P<0.01) * : Significant by Fisher exact test (P<0.05) **: Significant by Fisher exact test (P<0.01) 1) : No effect of *N, N*-dimethylformamide on organs other than the liver 2) : Hepatocellular adenoma or carcinoma Nonneoplastic changes other than altered hepatocyte are described in the repeated toxicity (Section 7.3.3).	
Rat BD 15 or 5 animals /group	Oral Drink-ing water	250 and 500 days	0, 75 mg/kg/day (for 500 days) 150 mg/kg/day (for 250 days) (total doses: 38 g/kg)	Mean surviving period: 532 days Carcinogenicity was not confirmed in this condition.	Druckrey et al., 1967
Rat BD 12 animals /group	Subcut aneous	104 or 109 weeks (once/week)	0, 200, 400 mg/kg/day (total dose: 8, 20 g/kg)	Carcinogenicity was not confirmed in this condition.	Druckrey et al., 1967

Table 7-9 Evaluations of carcinogenicity of *N, N*-dimethylformamide by the international and national organizations

Organization/ source	Classification	Classification criteria
IARC (2001)	Group 3	The agent is not classifiable as to carcinogenicity in humans
ACGIH (2001)	A4	Not classifiable as a human carcinogen.
The Japan Society for Occupational Health (2001)	Group 2B	The substance with less evidence (possibly carcinogenic to humans).
U.S. EPA (2002)	-	Not evaluated for human carcinogenicity.
U.S. NTP (2002)	-	Not evaluated for human carcinogenicity.

7.4 Summary of effects on human health

In humans and experimental animals, *N, N*-dimethylformamide is rapidly absorbed via oral, inhalation and dermal routes, and it is confirmed that *N, N*-dimethylformamide is transferred to fetuses.

As the acute effects of *N, N*-dimethylformamide to humans, irritation to the skin, eye, upper airway and gastrointestinal tract and effects on the liver are reported. In many of the long-term exposure studies, hepatic dysfunction was reported with the observation of blood biochemical changes, and diffuse hepatic degeneration and single cell necrosis in hepatic biopsy. Carcinogenicity of *N, N*-dimethylformamide has been reported, however, the causal relationship was not confirmed and the international institutions considered that evidences for carcinogenicity in human are insufficient.

The effects of *N, N*-dimethylformamide in experimental animals are summarized below.

In the acute toxicity of *N, N*-dimethylformamide to experimental animals, the oral LD_{50} is 3,700 to 6,800 mg/kg in mice and 2,000 to 7,600 mg/kg in rats, and the LC_{50} for inhalation exposure is 2,000 to 6,120 ppm (corresponding to 6,080 to 18,605 mg/m^3) in mice and 2,500 to 5,020 ppm (7,600 to 15,261 mg/m^3) in rats. The acute symptoms after *N, N*-dimethylformamide administration were body weight loss, restlessness, sensitiveness, hind-limb paralysis and hepatic damage.

The irritation of *N, N*-dimethylformamide was investigated in rabbits and irritation to eyes was observed but not to the skin. No reports on sensitization were obtained in this investigation.

With regard to the repeated dose toxicity of *N, N*-dimethylformamide, effects on the liver are found in all administration routes and common effects are the increse of liver weight, hepatic degeneration and necrosis and blood biochemical changes. Other than the liver, renal disorder and changes in cardiac function and myocardium were found. The NOAEL of *N, N*-dimethylformamide for oral administration is considered to be 200 ppm (corresponding to 17.2 mg/kg/day) based on the results of the 90-day oral feeding study of *N, N*-dimethylformamide in rats, and the LOAEL for inhalation exposure as 25 ppm (corresponding to 76 mg/m^3) based on the results of the 18-month inhalation exposure study in mice and the NOAEL as 25 ppm (corresponding to 76 mg/m^3) based on the results of the 2-year inhalation exposure study in rats.

With regard to the reproductive and developmental toxicity, *N, N*-dimethylformamide showed reproductive and developmental toxicty, and the NOAEL of *N, N*-dimethylformamide for oral administration is determined as 50 mg/kg/day based on the results of the teratogenicity study in rats, and

the NOAEL for inhalation exposure is determined as 32 ppm (corresponding to 97 mg/m^3) based on the results of the teratogenicity study in rats. The NOAEL of reproductive toxicity is determined as 50 mg/kg/day for oral administration, and 32 ppm (corresponding to 97 mg/m^3) for inhalation exposure.

N, N-dimethylformamide was negative in most of the *in vitro* genotoxicity studies including reverse mutation assays in *Salmonella typhimurium*, mutation and chromosomal aberration tests in human and Chinese hamster cultured cells and mutation aberration tests in mouse lymphoma. Of *in vivo* studies, negative results were obtained in a micronucleus test in mice and sex-linked recessive lethal test in *Drosophila*. The overall evaluation of the available data indicates that *N, N*-dimethylformamide is not genotoxic.

Regarding carcinogenicity of *N, N*-dimethylformamide, in an 18-month inhalation exposure study in ICR mice and a 2-year inhalation exposure studies in SD rats, no turmors were observed although preneoprastic effects on the liver were found in mice at 25 ppm and above and in rats at 100 ppm and above. It was reprted in 2004 that the hepatic tumors were developed in inhalation exposure studies in BDF_1 mice and F344 rats. *N, N*-dimethylformamide is considered carcinogenic to experimental animals in inhalation exposure.

N, N-dimethylformamide has been categorized as Group 3 (not classifiable as to its carcinogenicity to humans) by the IARC.

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ABBREVIATIONS

ACGIH : American Conference of Governmental Industrial Hygienists
ADH : Alcohol dehydrogenase
ALDH : Aldehyde dehydrogenase
ALP : Alkaline phosphatase
ALT : Alanine aminotransferase
ASAT : Aspartate aminotransferase
AST : Aspartate aminotransferase
ATSDR : Agency for Toxic Substances and Disease Registry
BCF : Bioconcentration Factor
BHK : Syrian hamster kidney culture cells
BOD : Biological Oxygen Demand
BUN : Blood urea nitrogen
CAS : Chemical Abstract Services
CAS Online : Chemical Abstract Services Online
CEPA : Commonwealth Environment Protection Agency
CERHR : Center for the Evaluation of Risks to Human Reproduction
CERI : Chemicals Evaluation and Research Institute, Japan
CHL : Chinese hamster lung cells
CHO : Chinese hamster ovary cells
CICAD : Concise International Chemical Assessment Document
Cmax : Maximum concentration of a compound in the blood, etc.
COD : Chemical Oxygen Demand
CPK : Creatinine phosphokinase
DDT : Dichlorodiphenyltrichloroethane
DOC : Dissolved Organic Carbon
EA : Environment Agency of Japan
EC : European Communities
EC_{10} : 10% Effect Concentration
EC_{50} : 50% Effect Concentration
ECB : European Chemicals Bureau
ECETOC : European Centre for Ecotoxicology and Toxicology of Chemicals
EEC : European Economic Communities
EHC : Environmental Health Criteria
EHI : Estimated Human Intake
EPA : Environmental Protection Agency (USA)
EU : European Union
EUSES : Europian Union System for the Evaluation of Substances
FAD : Flavin adenine dinucleotide
FAO : Food and Agriculture Organisation of the United Nations
GABA : g-Aminobutyric acid
GC : Gas chromatography
GGT : γ-Glutamyl transpeptidase
GLP : Good Laboratory Practice
hr : Hour
HSDB : Hazardous Substances Data Bank
IARC : International Agency for Research on Cancer
IC : Industrial Category
IC_{50} : 50% Immobilisation Concentration or 50% Inhibitory Concentration
ILO : International Labour Organisation
IPCS : International Programme on Chemical Safety
IRIS : Integrated Risk Information System
IUCLID : International Uniform Chemical Information Database (existing substances)
Koc : Soil adsorption coefficient Koc
Kow : Octanol/water partition coefficient
LC_{50} : Median Lethal Concentration

LD_{50} : Median Lethal Dose
LDH : Lactate dehydrogenase
LLNA : Local Lymph Node Assay
LOAEL : Lowest Observed Adverse Effect Level
LOEC : Lowest Observed Effect Concentration
LOEL : Lowest Observed Effect Level
MAO : Monoamineoxydase
MATC : Maximum Acceptable Toxic Concentration
MCH : Mean corpuscular hemoglobin
MCV : Mean corpuscular volume
METI : Ministry of Economy, Trade and Industry, Japan
MHLW : Ministry of Health, Labour and Welfare, Japan
min : Minute
MITI : Ministry of International Trade and Industry, Japan
MNLD : Maximum non lethal dose
MOE : Ministry of the Environment, Japan
MOF : Ministry of Finance, Japan
MOS : Margin of Safety
MTD : Maximum Tolerance Dose
NAT2 : *N*-Acetyltransferase
NCI : National Cancer Institute
NICNAS : Australia's National Industrial Chemicals Notification and Assessment Scheme
NIES : National Institute for Environmental Studies, Japan
NITE : National Institute of Technology and Evaluation, Japan
NMR : Nuclear magnetic resonance
NOAEL : No Observed Adverse Effect Level
NOEC : No Observed Effect Concentration
NOEL : No Observed Effect Level
NTE : Neurotoxic esterase
NTP : National Toxicology Program (USA)
NZW : New Zealand White
OECD : Organisation for Economic Cooperation and Development
OPIDN : Organophosphate-induced delayed neuropathy
OR : Odds ratios
ppm : Parts per million
$polA^-$: DNA polymerase$^-$
$polA^+$: DNA polymerase$^+$
pKa : Negative log of the acid dissociation constant
PRTR : Pollutant Release and Transfer Register
RBC : Radiation Biology Center
RAR : Risk Assessment Report
RC : Risk Characterisation
RfC : Reference Concentration
RfD : Reference Dose
RTECS : Registry of Toxic Effects of Chemical Substances
SCE : Sister chromatid exchange
SDH : Sorbitol dehydrogenase
SER : Smooth endoplasmic reticulum
SG : Syrian golden
SIDS : Screening Information Data Set
SLRL-test : Sex-linked recessive lethal test
SOD : Superoxide dismutase
TDI : Tolerable Daily Intake
TE : Toxic equivalent
TLV : Threshold Limit Value
Tmax : Time until a concentration reaches Cmax.
TOXLINE : Toxicology Literature Online
UV : Ultraviolet

v/v : volume per volume
w/w : weight per weight
WHO : World Health Organization
γ-GTP : γ-Glutamyl transpeptidase
δALS : δ-Aminolevulinic acid synthetase