SCREENING LEVEL HAZARD CHARACTERIZATION FOR HIGH PRODUCTION VOLUME CHEMICALS

SPONSORED CHEMICAL

N-Methylformamide (CAS No. 123-39-7)
[9th CI Name: Formamide, N-methyl-]

SUPPORTING CHEMICAL
Dimethylformamide (CAS No. 68-12-2)
[9th CI Name: Formamide, N, N'-dimethyl-]

December 2007
INTERIM

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SCREENING-LEVEL HAZARD CHARACTERIZATION OF HIGH PRODUCTION VOLUME CHEMICALS

The High Production Volume (HPV) Challenge Program[1] is a voluntary initiative aimed at developing and making publicly available screening-level health and environmental effects information on chemicals manufactured in or imported into the United States in quantities greater than one million pounds per year. In the Challenge Program, producers and importers of HPV chemicals voluntarily sponsor chemicals; sponsorship entails the identification and initial assessment of the adequacy of existing toxicity data/information, conducting new testing if adequate data do not exist, and making both new and existing data and information available to the public. Each complete data submission contains data on 18 internationally agreed to "SIDS" (Screening Information Data Set[1,2]) endpoints that are screening-level indicators of potential hazards (toxicity) for humans or the environment.

The Environmental Protection Agency's Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program on approximately 1400 sponsored chemicals. OPPT is using a hazard-based screening process to prioritize review of the submissions. The hazard-based screening process consists of two tiers described below briefly and in more detail on the Hazard Characterization website[3].

Tier 1 is a computerized sorting process whereby key elements of a submitted data set are compared to established criteria to "bin" chemicals/categories for OPPT review. This is an automated process performed on the data as submitted by the sponsor. It does not include evaluation of the quality or completeness of the data.

In Tier 2, a screening-level hazard characterization is developed by EPA that consists of an objective evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. The evaluation is performed according to established EPA guidance[2,4] and is based primarily on hazard data provided by sponsors. EPA may also include additional or updated hazard information of which EPA, sponsors or other parties have become aware. The hazard characterization may also identify data gaps that will become the basis for a subsequent data needs assessment where deemed necessary. Under the HPV Challenge Program, chemicals that have similar chemical structures, properties and biological activities may be grouped together and their data shared across the resulting category. This approach often significantly reduces the need for conducting tests for all endpoints for all category members. As part of Tier 2, evaluation of chemical category rationale and composition and data extrapolation(s) among category members is performed in accord with established EPA[2] and OECD[5] guidance.

The screening-level hazard characterizations that emerge from Tier 2 are important contributors to OPPT's existing chemicals review process. These hazard characterizations are technical documents intended to support subsequent decisions and actions by OPPT. Accordingly, the documents are not written with the goal of informing the general public. However, they do provide a vehicle for public access to a concise assessment of the raw technical data on HPV chemicals and provide information previously not readily available to the public. The public, including sponsors, may offer comments on the hazard characterization documents.

The screening-level hazard characterizations, as the name indicates, do not evaluate the potential risks of a chemical or a chemical category, but will serve as a starting point for such reviews. In 2007, EPA received data on uses of and exposures to high-volume TSCA existing chemicals, submitted in accordance with the requirements of the Inventory Update Reporting (IUR) rule. For the chemicals in the HPV Challenge Program, EPA will review the IUR data to evaluate exposure potential. The resulting exposure information will then be combined with the screening-level hazard characterizations to develop screening-level risk characterizations[4,6]. The screening-level risk characterizations will inform EPA on the need for further work on individual chemicals or categories. Efforts are currently underway to consider how best to utilize these screening-level risk characterizations as part of a risk-based decision-making process on HPV chemicals which applies the results of the successful U.S. High Production Volume Challenge Program and the IUR to support judgments concerning the need, if any, for further action.

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[1] U.S. EPA. High Production Volume (HPV) Challenge Program; http://www.epa.gov/chemrtk/index.htm.
[2] U.S. EPA. HPV Challenge Program – Information Sources; http://www.epa.gov/chemrtk/pubs/general/guidocs.htm.
[3] U.S. EPA. HPV Chemicals Hazard Characterization website (http://www.epa.gov/hpvis/abouthc.html).
[4] U.S. EPA. Risk Assessment Guidelines; http://cfpub.epa.gov/ncea/raf/rafguid.cfm.
[5] OECD. Guidance on the Development and Use of Chemical Categories; http://www.oecd.org/dataoecd/60/47/1947509.pdf.
[6] U.S. EPA. Risk Characterization Program; http://www.epa.gov/osa/spc/2riskchr.htm.

SCREENING LEVEL HAZARD CHARACTERIZATION
N-methylformamide (CAS No. 123-39-7)

Introduction

The sponsor, E. I. du Pont de Nemours & Co, submitted a Test Plan and Robust Summaries to EPA for N-Methylformamide also known as monomethylformamide (MMF) (CAS No. 123-39-7; 9th CI name: Formamide, N-methyl-) on March 30, 2004. EPA posted the submission on the ChemRTK HPV Challenge website on April 13, 2004 (http://www.epa.gov/chemrtk/pubs/summaries/monomthf/c15159tc.htm). EPA comments on the original submission were posted to the website on August 30, 2005. Public comments were also received and posted to the website. The sponsor submitted updated/revised documents on June 15, 2006 and December 13, 2007, which were posted to the ChemRTK website on September 5, 2006 and January 31, 2007, respectively.

This screening-level hazard characterization is based primarily on the review of the test plan and robust summaries of studies submitted by the sponsor(s) under the HPV Challenge Program. In preparing the hazard characterization, EPA considered its own comments and public comments on the original submission as well as the sponsor's responses to comments and revisions made to the submission. A summary table of SIDS endpoint data with the structure(s) of the sponsored chemical(s) is included in the appendix. The screening-level hazard characterization for environmental and human health toxicity is based largely on SIDS endpoints and is described according to established EPA or OECD effect level definitions and hazard assessment practices.

Justification for Supporting Chemical

For repeated-dose, reproductive and genetic toxicity endpoints, the sponsor indicated data on MMF are not available. The sponsor submitted data on an analog, dimethylformamide (DMF, CAS No. 68-12-2), to address these endpoints. The structure of DMF is closely related to MMF, in that both contain an N-substituted formamide moiety. The substances differ only in the degree of substitution on the nitrogen atom; MMF contains one methyl group and DMF contains two. As stated in the summary of the metabolism of MMF and DMF provided in the test plan, the pathways for biotransformation of DNF and MMF have been extensively studied and are qualitatively similar. The major pathway for metabolism of DMF is oxidation to form N(hydroxymethyl)-N-methylformamide and an alternative pathway is demethylation to MMF. Hepatotoxicity of both compounds is thought to be mediated by a reactive intermediate formed by further metabolism of MMF. EPA considered DMF to be a suitable analog for MMF based on similarities in their structures, physical-chemical properties and metabolism pathways showing similar target organ toxicity.

Summary-Conclusion

The log K_{ow} of monomethylformamide (MMF) indicates that its potential to bioaccumulate is expected to be low. Monomethylformamide is readily biodegradable, indicating that it is not expected to persist in the environment.

The evaluation of available toxicity data for fish, aquatic invertebrates and aquatic plants indicates that the potential acute hazard of MMF to aquatic organisms is low.

The acute oral and inhalation toxicity of MMF in rats and acute dermal toxicity in rabbits is low. MMF is irritating to rabbits eyes. In rats, repeatedly exposed to MMF via inhalation, body weights were reduced at a concentration of 0.32 mg/L/day or higher. Rats showed liver toxicity when exposed to the supporting chemical, DMF, via inhalation at concentrations of 1.2 mg/L/day and higher. Male mice exposed to DMF via inhalation showed no effects at the highest DMF concentration tested (2.4 mg/L/day); however, females had lengthened estrus cycles at 0.59 mg/L/day and higher. In a one-generation reproductive toxicity study in rats exposed dermally to the supporting chemical, DMF, excess mortality and decreased body weights were observed at high doses. At 1000 mg/kg-bw/day, effects on pups included smaller litter size, decreased viability to weaning, and, at the high dose only, delayed skeletal ossification. In developmental toxicity studies, MMF administered via gavage to rats and rabbits during organogenesis caused decreased maternal body weight gain and decreased food consumption. Offspring of both species exhibited gross malformations in both skeletal and visceral tissues including cephalocele, domed head,

flexed paw and gastroschisis. Developmental effects in rats exposed via inhalation at 0.4 mg/L/day included subcutaneous head cysts, microphthalmia, hydrocephaly, distended ventricles of the brain, fused ribs and vertebrae, and hemivertebrae. DMF also caused malformations when tested in rats and rabbits by various routes of exposure. DMF did not induce gene mutation or chromosomal aberrations. No carcinogenicity studies have been conducted with MMF. The International Agency for Research on Cancer (IARC) has reviewed the supporting chemical, DMF, for carcinogenicity and concluded that this chemical has been adequately characterized in rats and mice by the inhalation route of exposure showing no increase in tumor incidence above controls.

The potential health hazard of MMF is high based on the repeated-dose and reproductive/developmental toxicity. The carcinogenicity potential of the MMF analog, DMF, has been characterized in rats and mice by the inhalation route of exposure; showing no increase in tumor incidence above controls.

No data gaps were identified under the HPV Challenge Program.

1. Physical-Chemical Properties and Environmental Fate

A summary of physical-chemical properties and environmental fate data submitted is provided in Table 1. For the purpose of the screening-level hazard characterization, the review and summary of these data was limited to the octanol-water partition coefficient and biodegradation endpoints as indictors of bioaccumulation and persistence, respectively.

Octanol-Water Partition Coefficient

Log K_{ow}: -0.97 (estimated)

Biodegradation:

In the modified Sturm test using activated sludge as inoculum, 80% monomethylformamide degraded after 28 days. **Monomethylformamide is readily biodegradable.**

Conclusion: The log K_{ow} of monomethylformamide indicates that its potential to bioaccumulate is expected to be low. Monomethylformamide is readily biodegradable, indicating that it is not expected to persist in the environment.

2. Environmental Effects – Aquatic Toxicity

Acute Toxicity to Fish

(1) Fathead minnows (*Pimephales promelas*) were exposed to monomethylformamide at nominal concentrations of 0, 100, 1000 or 10,000 mg/L under static conditions for 96 hours. No mortality occurred at the highest concentration.
96-h LC_{50} > 10,000 mg/L

(2) Fathead minnows (*Pimephales promelas*) were exposed to monomethylformamide at nominal concentrations of 0, 0.5, 1.0, 50, 500 or 5000 mg/L under static conditions for 96 hours. No mortality occurred at the highest concentration. Rapid breathing was seen in all fish at the highest concentration.
96-h LC_{50} > 5000 mg/L

Acute Toxicity to Aquatic Invertebrates

Daphnia magna were exposed to monomethylformamide at nominal concentrations of 0, 62.5, 125, 250 or 500 mg/L under static conditions for 48 hours. No mortality/immobility occurred at any concentration.
48-h EC_{50} > 500 mg/L

Toxicity to Aquatic Plants

Green algae (*Scenedesmus subspicatus*) were exposed to monomethylformamide at nominal concentrations of 0, 5,000, 8,000, 12,800, 20,500, 32,800 or 42,400 mg/L under static conditions for 72 hours. No effect on growth was detected at concentrations of 8,000 mg/L or less.
96-h EC_{50} (biomass) = 17,300 mg/L

Conclusion: The evaluation of available toxicity data for fish, aquatic invertebrates and aquatic plants indicates that the potential acute hazard of monomethylformamide to aquatic organisms is low.

3. Human Health Effects

Acute Oral Toxicity

Female mice (Balb/c, number not reported) were administered monomethylformamide as single oral dose at 5 doses (doses not reported) and were observed for 30 days.
LD_{50} = 2600 mg/kg-bw

Acute Inhalation Toxicity

Male rats (Crl:CD, 6) were exposed to monomethylformamide vapor at concentrations of 0, 0.69, 1.0, 5.6 and 10.76 mg/L for 4 hours and were observed for an unspecified time after exposure. No deaths occurred but rats in the two highest exposure groups exhibited severe weight loss followed by recovery. Gasping was observed during exposure in 2 of 6 rats at the highest concentration.
LC_{50} > 10.76 mg/L

Repeated-Dose Toxicity

Monomethylformamide (MMF; CAS No. 123-39-7)
Male rats (15/dose) were exposed to monomethylformamide vapor via nose-only inhalation, at measured concentrations of 0, 0.12, 0.32, or 0.97 mg/L for 6 hours/day, 5 days/week for 2 weeks (10 exposures total). Five rats per group were sacrificed on day 14 of the study and five rats per group were held for a two-week recovery period. The remaining five rats per group were used solely for monomethylformamide analysis in urine. No mortality was reported at any exposure level. At 0.12 mg/L, no adverse effects were reported. At 0.32 mg/L, rats exhibited markedly decreased body weights during the two week exposure period. However, during the two-week recovery period, the body weight gains were comparable to controls. Relative liver weights and cholesterol were increased at this concentration. No adverse effects were reported following histopathological evaluation at the end of treatment. Following the two-week recovery period, no adverse effects were noted.

At 0.97 mg/L, rats exhibited markedly reduced body weights and increased cholesterol levels, aspartate aminotranspeptidase activity and alanine aminotranspetidase activity. In recovery animals, these parameters were unaffected. Increased relative liver and testes weights were seen at the end of the exposure, which returned to normal after 14 days recovery. Absolute weights of heart, lung, spleen, and thymus were decreased compared to controls at the end of the exposure period (robust summary did not include the evaluation of these organs during the recovery period). Histopathological evaluation of liver revealed degenerative/regenerative changes characterized as "pale cytoplasm", the presence of lipid vacuoles and increased mitotic figures.
LOAEL = 0.32 mg/L (based on decreased body weight)
NOAEL = 0.12 mg/L

Dimethylformamide (DMF; CAS No. 68-12-2, supporting Chemical)
Fischer 344 rats (30/sex/dose) and B6C3F1 mice (10/sex/dose) were administered the supporting chemical, dimethylformamide via whole-body inhalation at 0, 50, 100, 200, 400 and 800 ppm (approximately 0.15, 0.30, 0.60, 1.2, or 2.4 mg/L, respectively) for 6 hours/day, 5 days/week for 13 consecutive weeks.
Rats: No mortality occurred at any DMF concentration. Body weight gains were decreased the two highest exposure groups, markedly at the highest concentration. Clinical chemistry parameters measured on days 4, 24, and

91, revealed significantly ($p < 0.05$ and $p < 0.01$) elevated activities of alanine aminotransferase, sorbitol dehydrogenase, and isocitrate dehydrogenase in animals exposed to 200 ppm or higher by as early as day 4 of exposure. Serum cholesterol levels were significantly increased ($p < 0.01$) in all exposed rats at all time points. Liver weights were significantly ($p < 0.01$) increased at 100 ppm and higher in males and at all concentrations in females with slight to moderate centrilobular necrosis at 400 and 800 ppm (more severe in females). Relative testes weights were increased at 400 and 800 ppm; no microscopic findings or adverse effects on sperm density or motility were seen. Prolonged diestrus was noted in females exposed to 800 ppm.
For rats, NTP determined a LOAEL for DMF at 401 ppm based on liver toxicity, even though some clinical chemistry parameters indicative of liver injury were observed at lower exposure concentrations. In mice, the NTP report authors apparently did not consider liver changes (increased weights and hepatocyte size) as anything more than an adaptive response (i.e., mixed function oxidase induction) as there was no accompanying centrilobular necrosis when examined histologically, leaving a NOAEL greater than or equal to the highest exposure tested of 804 ppm. In female rats, the LOAEL is 200 ppm based on lengthened estrous cycles with a NOAEL of 50 ppm.
LOAEL (male) = 1.2 mg/L (400 ppm) (based on adverse effects on the liver**)**
NOAEL (male) = 0.60 mg/L (200 ppm)
LOAEL (female) = 0.60 mg/L (200 ppm) (based on lengthened estrous cycle)
NOAEL (female) = 0.30 mg/L (100 ppm)

Mice: No mortality occurred in any DMF exposure group. Female body weight gains were reduced compared to controls at 800 ppm. Absolute and relative liver weights (significant, $p < 0.01$) were increased in all mice exposed to DMF with accompanying slight hepatocyte hypertrophy in all mice exposed to 100 ppm DMF and higher and in males at 50 ppm. In females, a trend toward an increase in the estrus cycle length was seen. No effects on reproductive system of male mice were noted.
LOAEL (male) = 0.15 mg/L (50 ppm) (based on decreased liver weights)
NOAEL (male) = Not established
LOAEL (female) = 0.60 mg/L (200 ppm) (based on increased estrus cycle length)
NOAEL (female) = 0.30 mg/L (100 ppm)

Reproductive Toxicity

Dimethylformamide (DMF; CAS No. 68-12-2, supporting chemical)
(1) In a continuous breeding study, CD-1 mice (male and female) were administered DMF in the drinking water at 0, 1000, 4000, 7000 ppm (approximately 219, 820, and 1455 mg/kg-bw/day). The study consisted of 4 segments: (1) F0 cohabitation and lactation phase, (2) a crossover mating trial of the F0 generation, (3) a fertility assessment of the F1 generation and (4) reproductive performance of the second generation.

During F0 cohabitation and lactation phase, mice were housed in breeding pairs within dose groups. Litter interval, number, sex, weight of pups per litter, number of litters per breeding pair, and the post-natal day (PND) 0 body weights were monitored. F0 females were allowed to deliver and rear the final litter until PND 21. Pups were sexed, counted, and weighed periodically through the postnatal phase. On PND 21, randomly selected F1 pups from each dose group were weaned and housed in same-sex pairs by dose and saved for the F1 fertility assessment phase (described below). After completion of the cohabitation and lactation phase, all F0 animals were maintained on their respective treatment until scheduled sacrifice following the completion of the crossover mating phase.

There was no effect on survival, dose-related clinical signs of toxicity, body weight, food consumption or water consumption. Female body weight was significantly ($p < 0.05$) decreased at 7000 ppm at weeks 8 and 16. Maternal body weight decreased at all doses. Maternal food consumption was decreased during the lactation period. Reduced fertility was noted at 4000 ppm; and at 7000 ppm. The average number of litters per pair, average litter size, proportion of pups born alive, and average pup weight were all reduced compared to control pairs. Pups showed external malformations and other abnormalities, including domed head and hematomas along the nose and on the head. Those pups most severely affected died shortly after birth, and many were cannibalized prior to examination. The proportion of litters with one or more pups with an abnormal appearance was 7.9, 10.5, 90.0, and 77.8% for the 0, 1000, 4000, and 7000 ppm groups, respectively. The reduction in the proportions of litters with malformed pups in the high-dose group, compared to the mid-dose group, was influenced by the decreased fertility, increased prenatal death, and postnatal cannibalism observed in the high dose group. During the lactation phase, average post-natal survival was reduced in the 4000 and 7000 ppm groups.

For crossover mating, only the control and high-dose mice were used. Three breeding groups of F0 animals were created: 1) control male mated with control female, 2) high-dose male mated with control female, and 3) control male mated with high-dose female. No treatments were administered during cohabitation. Dosing was resumed after confirmation of pregnancy.

High-doe females treated with 7000 ppm produced fewer live pups per litter and pups with lower weights when compared to the females treated with 7000 ppm. However, males treated with 7000 ppm had an increased incidence of incomplete ossification of the cranial bones. Malformations observed in the control male mated with 7000 ppm-females included abnormal ossification of the cranial plates, abnormal suture formation in the cranium, and abnormal or incomplete formation of the sternebrae. Pups born to DMF-treated mothers had malformations, including agenesis of the cerebrum, agnathia, abnormally shaped centrum or cranium, cleft palate, or enlarged cerebral ventricles. The length of the estrous cycle was also affected by DMF. At necropsy, DMF-treated F0 females had significantly depressed body weights. Male liver weights and female absolute and relative liver weight, kidney and adrenal weights were increased at all doses. Caudal epididymal weight was significantly increased at all doses of DMF. A slight decrease in testicular spermatid concentration in the DMF-treated animals showed a significant ($p<0.01$) trend. However, no correlation of this finding was noted during the Histopathological examination of the reproductive organs. Therefore, the effect on testicular spermatids was not considered biologically relevant. Histopathological examination of mid-dose females and high-dose males revealed treatment-related centrilobular hepatocellular hypertrophy.

For F1 fertility assessment, randomly selected F1 pups from all groups began directly receiving DMF via the drinking water at PND 21. At 74±10 days of age, males and females in the control or treated groups were cohabited as nonsibling breeding pairs. Food consumption was unaffected in the F1 generation. Water consumption was increased for the males in the mid- and high-dose groups and body weights were reduced at these doses in both sexes. Selected F2 litters were preserved on PND 1 and evaluated for whole body skeletal malformations and soft tissue malformations of the head. Selected adult F1 males and females were evaluated for skeletal malformations. The survival of F1 pups in the final litter and postnatal survival on PND 4 was reduced at 4000 and 7000 ppm and continued to decline throughout the lactation period. Pup weight during lactation was reduced at the mid and high doses prior to PND7. The F1 pups at these doses also exhibited craniofacial malformations. Pups that were severely malformed did not survive the pre-weaning period. The surviving F1 pups were small and had foreshortened, domed heads. After weaning, pups were randomly selected for rearing and inclusion in the reproductive performance evaluation of the F1 generation.

During *reproductive performance evaluation of the second generation*, the following observations were made: The mating index was significantly ($p <0.05$) decreased at 7000 ppm. Fertility was reduced in the 4000 and 7000 ppm groups. The average days to litter was increased, and the number of live pups per litter, pup body weight, and the proportion of pups born alive was decreased in the 4000 and 7000 ppm groups. Live pup weight was also decreased in the 1000 ppm pups. F2 pups born to DMF-treated F1 pairs exhibited malformations similar to those observed for the F1 litters described above. The proportion of litters with 1 or more externally malformed pups was 0, 27.7, 60, and 75% for the 0, 1000, 4000 and 7000 ppm groups, respectively. Females in the 7000 ppm group had significantly longer estrus cycles and tended to be in either metestrus or diestrus longer than the control animals. At necropsy, F1 male and female body weights were reduced in the 4000 and 7000 ppm groups. Absolute and relative liver weights were markedly increased in all DMF-treated animals of both sexes. Female relative kidney plus adrenal weight was increased in the 4000 and 7000 ppm groups. Histopathological examination of the animals with gross lesions revealed treatment-related centrilobular hepatocellular hypertrophy. Absolute prostate weight was decreased in males at 4000 and 7000 ppm and relative prostate weight was decreased at all doses. Epididymidal spermatozoa concentration was decreased at 7000 ppm. Malformations observed in the mid- and high-dose animals consisted of abnormal or incomplete ossification of the cranial plates, abnormal cranial suture formation and abnormally formed sternebrae. Histopathological examination of additional F1 animals in the mid- and high-dose group revealed dysplasia of the cranial bones, primarily at the midline.

Significant reproductive and developmental toxicity was observed in both generations at 4000 and 7000 ppm DMF in the presence of some general toxicity. The liver appeared to be the primary non-reproductive target organ. Reduced F2 pup weight was noted at 1000 ppm DMF.
LOAEL (systemic toxicity) = 219 mg/kg-bw/day (based on mortality, decreased body weight and organ weights)
NOAEL (systemic toxicity) = Not established
LOAEL (reproductive toxicity) = 820 mg/kg-bw/day (based on decreased pup survival, decreased pup viability to weaning, increased estrus cycle, decreased prostate weights)
NOAEL (reproductive toxicity) = 219 mg/kg-bw/day
NOAEL (F1 offspring) = 219 mg/kg-bw/day (based on mortality and severe craniofacial malformations)
NOAEL (F2 offspring) = Not established

(2) In the previously described 13-week repeated-exposure inhalation studies with DMF in rats and mice, DMF did not affect the male reproductive organs but estrus cycles were prolonged in females exposed to 800 ppm (2.4 mg/L/day) in rats and 200 ppm (0.6 mg/L/day) in mice.

Developmental Toxicity

Monomethylformamide (MMF; CAS No. 123-39-7)
(1) Pregnant Crl:CD, BR rats (25 /group) were administered MMF by gavage at doses of 0, 1, 5, 10, or 75 mg/kg-bw/day during gestation days 6 through 15. Significantly decreased food consumption and decreased maternal weight gain ($p < 0.01$) were observed in the high-dose group. There was no effect on the number of corpora lutea or fetal sex ratios. Post-implantation loss was significant ($p < 0.01$), fetal viability was reduced due to a marked increase early resorptions and fetal weights were significantly decreased ($p < 0.01$) at 75 mg/kg-bw/day. Over 50% of the fetuses at 75 mg/kg-bw/day were malformed. Malformations included cephalocele (protrusion of a part of the cranial contents) and sternoschisis (cleft sternum) and skeletal variations included incomplete ossification of various skeletal structures including reduced ossification of the skull, 13th rib and sternebrae. Malformations seen at 10, 5, and 1 mg/kg-bw/day were not dose dependent or were within the historical control range. No malformations occurred in the pups from the control or 1 mg/kg-bw/day dose groups. One pup (from one litter) was malformed at 5 mg/kg-bw/day, 3 pups had malformations (from 3 different litters) at 10 mg/kg-bw/day and 150 pups showed malformations (from 21 affected litters) at75 mg/kg-bw/day. Malformations consisted primarily of cephalocele and sternoschisis.
LOAEL (maternal toxicity) = 75 mg/kg-bw/day (based on decreased weight gain and decreased food consumption)
NOAEL (maternal toxicity) = 10 mg/kg-bw/day
LOAEL (developmental toxicity) = 75 mg/kg-bw/day (based on gross malformations and skeletal variations)
NOAEL (developmental toxicity) = 10 mg/kg-bw/day

(2) Pregnant New Zealand White rabbits (20/group) were administered MMF via gavage at 0, 5, 10 or 50 mg/kg-bw/day during gestation days 6 through 18. There were no treatment-related maternal deaths, or effects on clinical signs or gross pathology. Body weight and food consumption were decreased and there was a statistically significant increase ($p < 0.05$) in post-implantation loss at 50 mg/kg-bw/day. The number of implantations and sex ratio at lower doses were unaffected by treatment. Fetal viability was reduced at 50 mg/kg-bw/day. Significant ($p < 0.05$) treatment-related significant malformations observed at 50 mg/kg-bw/day included cephalocele, domed head, bent hyoid, other skull and sternum anomalies, flexed paw, and gastroschisis. No increase in pup malformations was observed at 5 or 10 mg/kg-bw/day.
LOAEL (maternal toxicity) = 50 mg/kg-bw/day (based on decreased weight gain and decreased food consumption)
NOAEL (maternal toxicity) = 10 mg/kg-bw/day
LOAEL (developmental toxicity) = 50 mg/kg-bw/day (based on gross malformations and decreased survival)
NOAEL (developmental toxicity) = 10 mg/kg-bw/day

(3) Pregnant rats (Crl:CD BR, 25/group) were exposed (nose-only) to MMF vapor at concentrations of 0, 15, 50 or 150 ppm (approximately 0.04, 0.12 and 0.40 mg/L/day, respectively), 6 hours/day during gestation days 7 through 16. Dams in the mid- and high-exposure groups exhibited mild respiratory distress characterized as rales and wheezing. At 150 ppm, decreased food consumption, decreased body weight gain and decreased thymus weights were observed. Dams at this exposure level also showed increased resorptions. The mean number of live fetuses

per litter was also decreased. Pups from the 50 and the 150 ppm exposure groups had reduced fetal body weights. At 150 ppm, pups had the following malformations: subcutaneous head cysts, microphthalmia, hydrocephaly, distended ventricles of the brain, fused ribs and vertebrae, and hemivertebrae. An increased incidence of skeletal anomalies and retarded bone ossification also were evident in pups from the 150 ppm group.
LOAEL (maternal toxicity) = 0.12 mg/L/day (based on respiratory distress)
NOAEL (maternal toxicity) = 0.04 mg/L/day
LOAEL (developmental toxicity) = 0.12 mg/L/day (based on fetal body weight depression)
NOAEL (developmental toxicity) = 0.04 mg/L/day (15 ppm)

Other developmental toxicity studies on the supporting chemical, dimethylformamide (DMF), in rats and rabbits by oral and/or inhalation routes of exposure support the conclusion that MMF is a developmental toxicant.

Genetic Toxicity – Gene Mutation

In vitro

Monomethylformamide (MMF; CAS No. 123-39-7)
No reliable in vitro gene mutation tests were submitted for MMF.

Dimethylformamide (DMF; CAS No. 68-12-2, supporting chemical)
(1) *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to DMF, at concentrations of 0, 9400, 24000, 47000, 94000, 190000 or 470,000 μg/plate in the presence or absence of metabolic activation. The two highest concentrations were cytotoxic in the presence and absence of metabolic activation. Both positive and negative controls response was appropriate.
DMF was not mutagenic in this assay.

(2) The National Toxicology Program reports four mouse lymphoma studies, three negative and one positive for the supporting chemical DMF.

Genetic Toxicity – Chromosomal Aberration

In vitro
Monomethylformamide (MMF; CAS No. 123-39-7)
No *in vitro* chromosome aberration tests have been performed with MMF.

Dimethylformamide (DMF; CAS No. 68-12-2, supporting chemical)
The supporting chemical, DMF, was examined, no increase in chromosome aberrations or sister chromatid exchanges were detected in Chinese hamster ovary cells in two trials at concentrations ranging from 50 to 5000 μg/mL in the presence or absence of metabolic activation. Positive and negative controls gave appropriate responses.
DMF did not induce chromosomal aberrations in this assay.

In vivo
Monomethylformamide (MMF; CAS No. 123-39-7)
No *in vivo* chromosomal aberration studies with MMF have been conducted.

Dimethylformamide (DMF; CAS No. 68-12-2, supporting chemical)
A micronucleus assay was conducted with DMF in male BALB/c mice (5/dose), administered via intraperitoneal injection at doses of 0, 0.2, 20 or 2000 mg/kg-bw. Whether animals received more than one dose was not reported and other methodological details were absent from the robust summary. The positive and negative controls gave appropriate responses.
DMF did not induce chromosomal aberrations in this assay.

Genetic Toxicity – Other DNA Effects

In Vivo
Dimethylformamide (DMF; CAS No. 68-12-2, supporting chemical)
In the dominant lethal assay, rats (10/group) were exposed to dimethylformamide via the inhalation route of exposure 6 hours/day, for 5 days at concentrations of 0, 30, or 300 ppm. Individual males were housed with 2 untreated females for two weeks before treatment. Immediately after the treatment phase, males were housed with two new untreated females for two weeks, then two more untreated females. This was repeated over the 6-week cycle of sperm development in order to assess all phases of spermatogenesis. Females were sacrificed 18 days after the first day of caging and implantation sites were counted to assess dominant lethality. DMF did not affect implantation rates or increase fetal deaths indicating a negative response for dominant lethality. The positive control responded appropriately.
DMF was negative in this assay.

Additional Information

Eye Irritation

MMF (100 µl) was instilled into the lower conjunctival sac of one eye of six New Zealand White rabbits with the opposite eye as control. Effects on the cornea, iris and conjunctivae were evaluated according the scoring criteria of Draize at 4, 24, 48, 72, and 96 hours. Corneal opacity reached a severity of 1 (on a scale of 0 to 4) at the 4-hour observation point, after which scores diminished to 0.3 at the 72 hour observation period and clearing almost completely by 96 hours. Observation after 96 hours (conjunctivitis) was still persisted. MMF was irritating to rabbits' eyes.

Carcinogenicity

No carcinogenicity studies have been conducted with MMF. The International Agency for Research on Cancer (IARC) has reviewed the supporting chemical, DMF, for carcinogenicity and concluded that this chemical has been adequately characterized in rats and mice by the inhalation route of exposure showing no increase in tumor incidence above controls.

Conclusion: The acute oral and inhalation toxicity of MMF in rats and acute dermal toxicity in rabbits is low. MMF is irritating to rabbits eyes. In rats, repeatedly exposed to MMF via inhalation, body weights were reduced at a concentration of 0.32 mg/L/day or higher. Rats showed liver toxicity when exposed to the supporting chemical, DMF, via inhalation at concentrations of 1.2 mg/L/day and higher. Male mice exposed to DMF via inhalation showed no effects at the highest DMF concentration tested (2.4 mg/L/day); however, females had lengthened estrus cycles at 0.59 mg/L/day and higher. In a one-generation reproductive toxicity study in rats exposed dermally to the supporting chemical, DMF, excess mortality and decreased body weights were observed at high doses. At 1000 mg/kg-bw/day, effects on pups included smaller litter size, decreased viability to weaning, and, at the high dose only, delayed skeletal ossification. In developmental toxicity studies, MMF administered via gavage to rats and rabbits during organogenesis caused decreased maternal body weight gain and decreased food consumption. Offspring of both species exhibited gross malformations in both skeletal and visceral tissues including cephalocele, domed head, flexed paw and gastroschisis. Developmental effects in rats exposed via inhalation at 0.4 mg/L/day included subcutaneous head cysts, microphthalmia, hydrocephaly, distended ventricles of the brain, fused ribs and vertebrae, and hemivertebrae. DMF also caused malformations when tested in rats and rabbits by various routes of exposure. DMF did not induce gene mutation or chromosomal aberrations. No carcinogenicity studies have been conducted with MMF. The International Agency for Research on Cancer (IARC) has reviewed the supporting chemical, DMF, for carcinogenicity and concluded that this chemical has been adequately characterized in rats and mice by the inhalation route of exposure showing no increase in tumor incidence above controls.

The potential health hazard of MMF is high based on the repeated-dose and reproductive/developmental toxicity. The carcinogenicity potential of the MMF analog, DMF, has been characterized in rats and mice by the inhalation route of exposure; showing no increase in tumor incidence above controls.

4. Hazard Characterization

The log K_{ow} of monomethylformamide (MMF) indicates that its potential to bioaccumulate is expected to be low. Monomethylformamide is readily biodegradable, indicating that it is not expected to persist in the environment.

The evaluation of available toxicity data for fish, aquatic invertebrates and aquatic plants indicates that the potential acute hazard of MMF to aquatic organisms is low.

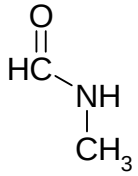
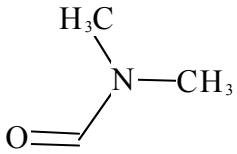
The acute oral and inhalation toxicity of MMF in rats and acute dermal toxicity in rabbits is low. MMF is irritating to rabbits eyes. In rats, repeatedly exposed to MMF via inhalation, body weights were reduced at a concentration of 0.32 mg/L/day or higher. Rats showed liver toxicity when exposed to the supporting chemical, DMF, via inhalation at concentrations of 1.2 mg/L/day and higher. Male mice exposed to DMF via inhalation showed no effects at the highest DMF concentration tested (2.4 mg/L/day); however, females had lengthened estrus cycles at 0.59 mg/L/day and higher. In a one-generation reproductive toxicity study in rats exposed dermally to the supporting chemical, DMF, excess mortality and decreased body weights were observed at high doses. At 1000 mg/kg-bw/day, effects on pups included smaller litter size, decreased viability to weaning, and, at the high dose only, delayed skeletal ossification. In developmental toxicity studies, MMF administered via gavage to rats and rabbits during organogenesis caused decreased maternal body weight gain and decreased food consumption. Offspring of both species exhibited gross malformations in both skeletal and visceral tissues including cephalocele, domed head, flexed paw and gastroschisis. Developmental effects in rats exposed via inhalation at 0.4 mg/L/day included subcutaneous head cysts, microphthalmia, hydrocephaly, distended ventricles of the brain, fused ribs and vertebrae, and hemivertebrae. DMF also caused malformations when tested in rats and rabbits by various routes of exposure. DMF did not induce gene mutation or chromosomal aberrations. No carcinogenicity studies have been conducted with MMF. The International Agency for Research on Cancer (IARC) has reviewed the supporting chemical, DMF, for carcinogenicity and concluded that this chemical has been adequately characterized in rats and mice by the inhalation route of exposure showing no increase in tumor incidence above controls.

The potential health hazard of MMF is high based on the repeated-dose and reproductive/developmental toxicity. The carcinogenicity potential of the MMF analog, DMF, has been characterized in rats and mice by the inhalation route of exposure; showing no increase in tumor incidence above controls.

5. Data Gaps

No data gaps were identified under the HPV Challenge Program.

APPENDIX

Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program		
Endpoints	**SPONSORED CHEMICAL** **Monomethylformamide** **123-39-7**	**SUPPORTING CHEMICAL** **Dimethylformamide[1]** **68-12-2**
Structure		
Summary of Physical-Chemical Properties and Environmental Fate Data		
Melting Point (°C)	-3.8	-61
Boiling Point (°C)	199.5	152.5 – 153.5
Vapor Pressure (hPa at 25°C)	0.337 hPa	—
Log K_{ow}	-0.97 (measured)	-0.85 (measured)
Water Solubility (mg/L at 25°C)	1.0×10^{6}	2.0×10^{5}
Direct Photodegradation	Direct photolysis is not expected.	—*
Indirect (OH^{-}) Photodegradation Half-life ($t_{1/2}$)	57 hours	—*
Stability in Water (Hydrolysis) ($t_{1/2}$)	Note expected to hydrolyze in the environment due to very slow hydrolysis of amides under environmental conditions.	—*
Fugacity (Level III Model) **Air (%)** **Water (%)** **Soil (%)** **Sediment (%)**	 0.42 39.9 59.6 0.07	—*
Biodegradation at 28 days (%)	80 Readily biodegradable	—*
Summary of Environmental Effects – Aquatic Toxicity Data		
Fish 96-h LC_{50} (mg/L)	> 10000	—*
Aquatic Invertebrates 48-h EC_{50} (mg/L)	> 5000	—*
Aquatic Plants 72-h EL_{50} (mg/L) (growth and biomass)	> 8000	—*

— indicates endpoint was not addressed for this chemical; * indicates endpoint not required for this chemical

Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program		
Endpoints	**SPONSORED CHEMICAL** **Monomethylformamide** **123-39-7**	**SUPPORTING CHEMICAL** **Dimethylformamide[1]** **68-12-2**
Summary of Human Health Data		
Acute Oral Toxicity **LD_{50} (mg/kg-bw)**	4000 – 7077 (rat) 2600 (mouse)	—*
Acute Dermal Toxicity **LD_{50} (mg/kg-bw)**	11,000 (rat); 1500 (rabbit)	—*
Acute Inhalation Toxicity **LC_{50} (mg/L)**	> 10.8 (rat, 4-hr)	—*
Repeated-Dose Toxicity **NOAEL/LOAEL** **Inhalation (mg/L/day)** **Rat** **Mice**	 NOAEL = 0.12 (10-d) LOAEL = 0.32 (10-d)	 NOAEL (male) = 0.59 LOAEL (male) = 1.2 NOAEL (female) = 0.30 LOAEL (female) = 0.59 NOAEL (male) = None established LOAEL (male) = 0.15 NOAEL (female) = 0.30 LOAEL (female) = 0.59
Reproductive Toxicity **NOAEL/LOAEL (mg/kg-bw/day)** **Systemic Toxicity** **Reproductive Toxicity** **Offspring Toxicity**	No Data NOAEL = Not established LOAEL = 219 (RA) NOAEL = 219 LOAEL = 820 (RA) NOAEL = Not established LOAEL = 219 (RA)	 NOAEL = Not established LOAEL = 219 NOAEL = 219 LOAEL = 820 NOAEL = Not established LOAEL = 219
Developmental Toxicity **Oral NOAEL/LOAEL(mg/kg-bw/day)** **Maternal/Developmental Toxicity** **Maternal/Developmental Toxicity**	 (Rat) NOAEL = 10 LOAEL = 75 (Rabbit) NOAEL = 10 LOAEL = 50	—*
Developmental Toxicity **Inhalation (mg/L/day)** **Maternal/Developmental Toxicity**	 NOAEL = 0.04 LOAEL = 0.12	—*

— indicates endpoint was not addressed for this chemical; * indicates endpoint not required for this chemical

[1] Supporting chemical data was used for addressing the repeated-dose, reproductive and genetic toxicity endpoints.

Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program		
Endpoints	**SPONSORED CHEMICAL Monomethylformamide 123-39-7**	**SUPPORTING CHEMICAL Dimethylformamide[1] 68-12-2**
Genetic Toxicity – Gene Mutation ***In vitro***	No Data Negative (RA)	Negative
Genetic Toxicity – Chromosomal Aberrations ***In vivo***	No Data Negative (RA)	Negative
Genetic Toxicity – Chromosomal Aberrations ***In vitro***	No Data Negative (RA)	Negative
Genetic Toxicity – Other Dominant Lethal	No Data Negative (RA)	Negative
Additional Information Eye Irritation	Irritating	—*
Additional Information Carcinogenicity	—*	DMF has been adequately characterized in rats and mice by the inhalation route of exposure showing no increase in tumor incidence above controls (IARC).

— indicates endpoint was not addressed for this chemical; * indicates endpoint not required for this chemical

[1] Supporting chemical data was used for addressing the repeated-dose, reproductive and genetic toxicity endpoints.