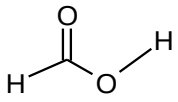
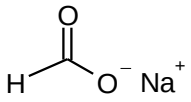
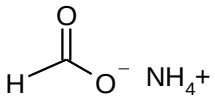
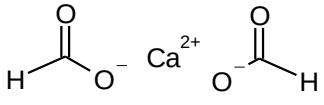
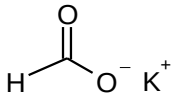
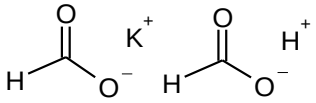
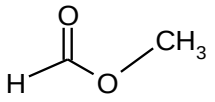
SIDS INITIAL ASSESSMENT PROFILE

Chemical category	Formic acid and Formates
Category Members: **CAS Registry Numbers and Chemical Names**	64-18-6 Formic acid (FoA) 141-53-7 Sodium formate (NaFo) 540-69-2 Ammonium formate (AFo) 544-17-2 Calcium diformate (CaFo) 590-29-4 Potassium formate (KFo) 20642-05-1 Potassium hydrogen diformate (KHFo) 107-31-3 Methyl formate (MeFo)
Category Members: **Structural Formulas**	64-18-6  141-53-7  540-69-2  544-17-2  590-29-4  20642-05-1  107-31-3 

SUMMARY CONCLUSIONS OF THE SIAR

CATEGORY/SUPPORTING CHEMICAL JUSTIFICATION

The sponsored Formates Category consists of FoA, five of its salts, and MeFo. The salts dissociate immediately in aqueous and biological surroundings to the formate ion. With a pKa of 3.7, FoA will also exist almost entirely as the formate ion at pH 7. It is therefore expected that the toxicological profiles of the acids and salts will be similar. Ammonium has already been assessed in the OECD HPV Chemicals Programme as ammonium chloride (CAS. 1212-502-9) and the hazard assessment is published:

[http://webnet.oecd.org/Hpv/UI/SIDS_Details.aspx?id=78FA6AC9-ADF3-4A43-894E-7D632A5BFFC3].

MeFo is included in the category for human health because it is enzymatically hydrolyzed to formate. However, it is also biotransformed to methanol and therefore must be treated somewhat differently from the other category members.

For aquatic toxicity endpoints, the category is appropriate for formic acid and its salts because salts dissociate in water to formic acid and counter ions. However, MeFo is handled separately from the other category members for ecological effects because it is an ester and does not dissociate in water with a hydrolysis half life of 67 hours (2.9 days) at pH 7 and 20 °C. Therefore, MeFo is not used to read across to other category members.

Subcategory I: Formic acid and its dissociative salts

FoA is a volatile, strong acid (pH<2) and its acute toxicity and irritation to skin, eyes and respiratory tract is caused by local toxicity. The dissociative salts are less irritating. The category member cations (sodium-, potassium-, potassiumhydrogen-, calcium- and ammonium) are present in the environment and body fluids. They are not likely to influence the toxicity of the formate ion; therefore, reading across is possible among this subcategory.

Neither FoA nor the KH-salt were skin sensitizers; so reading across to the other salts is possible as also none of the cations are known to be skin sensitizers.

Studies on repeated exposure (subchronic to chronic toxicity effects, developmental toxicity and effects on fertility) were predominantly performed on dissociative salts to elucidate the toxicity of the formate ion. Tests with formic acid would obscure this effect due to the caustic nature of the acid. However, evaluation for worker safety requires inhalation toxicity testing of the acid for which data were available. Read across of cations is possible as none of them are known to cause toxic effects after repeated oral exposure or with respect to developmental toxicity or fertility. While data on KHFo were generated in the course of an animal feed additive registration, testing of NaFo was performed to address fertility via the oral route.

In vitro mutagenicity testing requires testing of chemicals at physiological pH values which is routinely done by neutralization in culture mediums containing the dissociative salts as nutritional parameters for the respective bacterial or mammalian cells. As none of the cations is known to be mutagenic, read across between the salts and the acid seems possible. This is also applicable to *in vivo* studies.

Subcategory II: Methylformate

As the methylformate ester does not hydrolyze rapidly in aqueous medium, it is not fully comparable to the dissociative salts. However, as it is effectively, rapidly cleaved by esterases in body fluids and even in nasal tissue as evident in a rat inhalation study with similar local toxicity of the acid and the ester, a subcategory was determined.

Data were available on acute toxicity, irritation (skin, eye and respiratory tract), mutagenicity (Ames Test) and repeated inhalation exposure of MeFo. Data for effects on fertility, developmental toxicity and mutagenicity (mammalian cell systems) from the cleavage products of methylformate, formate and methanol, were bridged. Methanol was evaluated in the OECD SIDS program (SIAM 19).

The availability of valid toxicity permitting read across is summarized as follows.

Endpoint	**FoA**	**Salts**[§]	**MeFo**
Acute toxicity	✓	✓	✓
Skin irritation	✓	✓	✓

Eye irritation	✓	✓	✓
Sensitization	✓	✓	⊗
Repeated toxicity	✓	✓	✓
Development	O	✓	O
Fertility	O	✓	⊗
Genotoxicity			
Bacteria	✓	✓	✓
Mammalian	O	✓	⊗

§ = data for at least one salt available

✓ = valid data available **O = read across from formates** **⊗ = read across from methanol / formates**

Local effects: With respect to human health, **local effects** have been observed following contact with FoA based on its acidity. KHFo exhibited eye and respiratory irritation, which is expected because it liberates FoA in aqueous solution; the proportion of FoA would be 35% on a weight basis. MeFo is hydrolyzed by esterases after inhalation (with a half life of 6 seconds at the site of exposure) and causes damage to the upper respiratory tract similar to formic acid.

Systemic effects: Systemic mammalian effects are expected to rely on the formate ion, which is common to FoA and its salts and is one of the breakdown products of MeFo. Therefore, data for the acid and salts are used in this assessment to read across to other category members. The toxicity of the counter ions of the salts is well understood. Sodium, calcium, and potassium occur in body fluids, and the toxicity of simple ammonium salts has been reviewed at previous SIAMs (Ammonium chloride at SIAM 17, and Ammonium sulfate at SIAM 19). As noted above, **the mammalian toxicity of MeFo** is also related to methanol, which is formed in equimolar amounts with formate. Therefore, as support for MeFo, methanol (CAS No. 67-56-1) data (approved at SIAM 19) are included for repeated-dose, chromosomal aberrations, and reproductive/developmental toxicity endpoints.

HUMAN HEALTH

Toxicokinetics, Metabolism, and Distribution: Formate is the common metabolite of all category chemicals. Formate is formed from precursors in the intermediary metabolism and is used as an important constituent of the C_1 intermediary metabolism which is required for the biosynthesis of amino acids and nucleic acid bases (purines and pyrimidines). Formate may also be formed from ingested methanol via formaldehyde and further oxidation to formate. Pharmacokinetic models have been established from methanol inhalation studies which allow calculating the time course of all metabolites including formate in good correlation with animal studies. Peak plasma formate levels were reached within 1 hour (rabbits) and 4-5 hours (pigs) after oral administration of KHFo. The elimination from blood follows first order kinetics and the blood levels rapidly return to background levels in all species, i.e. formate does not persist or accumulate. However, there are significant species differences in the elimination rates and the elimination half-lives (from plasma): rat (12 minutes) < guinea pig (22 minutes) < rabbit (32 minutes) < humans (45 minutes) < cat (67 minutes) < dog (77 minutes) < pig (87 minutes). This reflects the species differences in the hepatic concentrations of folates and folate-dependent enzymes which affect the formate degradation to CO_2. Only minor quantities are excreted unchanged via urine in all species.

All category members may be absorbed via the oral route. FoA and MeFo may generate vapors that can be takn up by inhalation.

Subcategory I: Formic acid and its dissociative salts

Acute Toxicity

The acute **inhalation** 4-h LC_{50} value for **FoA** vapor in rats was 7.4 mg/L in a study conducted comparable to OECD TG 403. Clinical signs in all treated groups included closed eyelids, discharge and corrosion of the nose and eye, salivation, corneal opacity, loss of pain reflex, dyspnea, noisy breathing, apathy, hunched posture, unsteady gait, and decreased body weight. Dead animals had dilated and hyperemic hearts and inflated lungs.

An LC_{50} of > 5.16 mg/L was determined for **KHFo** in a study conducted according to OECD TG 403. Clinical signs included strongly decreased breathing rates in all rats throughout exposure, piloerection, moderate sluggishness in all animals, rales, and blepharospasms. Lower body weight gain and changes in lungs (bleeding/discoloration) and intestines were seen.

An LC_{50} of > 0.67 mg/L for **NaFo** in a study similar to OECD TG 403 was associated with decreased activity and eye closing, lacrimation and nasal discharge, and a slight and transient reduction in body weight gain.

FoA was not tested for **dermal toxicity** due to its caustic nature (pH < 2). An LD_{50} of > 2000 mg/kg bw was obtained following 24-hour dermal exposure of rats to **NaFo** under semi-occlusive conditions (OECD TG 402). No mortality, clinical signs of toxicity, skin reactions or effects on body weight were noted. No changes in any organs were noted during necropsy.

The acute **oral** LD_{50} of **FoA** in the rat was 730 mg/kg bw (OECD TG 401). Severe clinical signs were noted approximately 30 minutes after dosing and included hunched posture, dyspnea, bloody nose and blood in urine. Gross pathology revealed hyperemia of the stomach and mottled livers and kidneys. An LD_{50} of > 2000 mg/kg bw in rats was determined for **AFo** in an OECD TG 423 study. No deaths occurred. Piloerection and hunched posture were seen immediately following dosing without pathological changes at termination. An LD_{50} of 3050 mg/kg bw was determined for **CaFo** in a study that was similar to OECD TG 401; clinical signs included sedation, increased diuresis, and reduced general state. An LD_{50} > 2000 mg/kg bw in rats was determined for **KHFo** in an OECD TG 401 study; clinical signs included lethargy, piloerection and tachypnea.

Studies using rats were not available for **NaFo** or **KFo**, but mouse LD_{50} s were 11200 mg/kg bw and 5500 mg/kg bw, respectively.

Overall, category members exhibit low acute toxicity via inhalation, dermal and oral routes.

Irritation/Sensitisation

No animal studies were available **for skin irritation** for **FoA**. However, in agreement with the low pH (<2), it is known that **FoA** is corrosive to the skin and gastro-intestinal tract in humans. **CaFo (**OECD TG 404) and **KHFo** (OECD TG 404) were not irritating to the skin.

FoA is assumed to be corrosive to the **eyes** due to its inherent properties as a strong acid and does not require testing. **NaFo** caused transient irritation of the rabbit's eye conjunctivae in a study that was conducted according to US EPA OTS guidelines. **CaFo** caused irritation to the rabbit's eye which was reversible within 13 days (OECD TG 405). **KHFo** was corrosive to the eyes both as a solid and as a 50 % aqueous solution (OECD TG 405).

FoA showed signs consistent with respiratory tract irritation in acute inhalation toxicity studies (OECD TG 403). **KHFo** caused sensory irritation in the respiratory tract in mice when tested according to Alarie's method.

FoA and KHFo were not sensitizing to skin when tested in the Buehler and the Guinea pig maximization tests, respectively, both according to OECD TG 406.

Conclusion: FoA is corrosive to human skin and assumed to be corrosive to eyes (based on strong acidic properties) while the salts of formic acid are neither corrosive nor irritating to skin. NaFo and CaFo showed transient eye irritation while KHFo was corrosive to rabbit eyes. FoA and KHFo showed respiratory tract irritation. The category members FoA and KHFo are not dermal sensitizers.

Repeated-Dose Toxicity

The studies in rodents that are described below must be interpreted with caution because rodents have high tetrahydrofolate and 10-formyl tetrafolate dehydrogenase levels, which allows them to rapidly metabolize formate to CO_2. Humans have much lower levels of this coenzyme and enzyme and therefore, might be more sensitive to formate.

FoA was evaluated in 13-week inhalation studies in rats and mice, and **KHFo** was tested in 13-week and 104-week rat and 80-week mouse dietary studies.

In an OECD TG 413 test, rats were exposed to **FoA** vapor at 0, 0.015, 0.030, 0.062, 0.122, or 0.244 mg/L (0, 8, 16, 32, 64, or 128 ppm) via whole-body inhalation 6 hours/day, 5 days/week for 13 weeks. Increased absolute or relative liver weights and decreased lung weights were seen without histopathological correlation. Irritation of the upper respiratory tract was seen at 128 ppm (0.244 mg/L) along with degeneration of the olfactory epithelium and squamous metaplasia of the respiratory epithelium. The NOAEC and LOAEC were 0.122 mg/(L*d) (i.e. 64 ppm) and 0.244 mg/(L*d) (i.e. 128 ppm) based on respiratory effects.

A 13-week study (OECD TG 413) in B6C3F1 mice with **FoA** using the same protocol and concentration as those used in the rat study, resulted in decreased body weights and increased liver weights in males at all doses. Mild degeneration of the olfactory epithelium was seen at the two highest concentrations. Based on the changes in olfactory epithelium, the NOAEC and LOAEC were determined to be 0.062 mg/(L*d) (32 ppm) and 0.122 mg/(L*d) (64 ppm), respectively.

KHFo was administered to rats in a 13-week oral feed study (OECD TG 408) at 0, 600, 1200, and 3000 mg/kg bw/d, followed by a 4-week recovery period. In males, body weight gain was decreased in a dose-dependent manner. Increased red blood cells, white blood cells, and platelets were seen in males at the highest dose, and additionally several changes in clinical chemistry were observed. Most treatment-related hematological and clinical chemical changes subsided within the recovery period. Adrenal weights were decreased and liver weights increased in both sexes. Thickening of stomach walls was increased in a dose-dependent manner in both sexes. Squamous cell hyperplasia of the forestomach correlated with thickening of stomach walls in all treated groups. Only high-dose animals still showed low incidences of mild hyperplasia at the end of the recovery period. Based on the changes in the stomach, no NOAEL could be established and the LOAEL was 600 mg/kg bw/d.

In a 104-week dietary study comparable to OECD TG 453, **KHFo** was administered to rats, at 0, 50, 400, and 2000 mg/kg bw/d. Body weight was consistently lower at the highest dose, accompanied by slight decreases in food consumption. Urea was increased in high-dose males. Macroscopically, increased incidences of raised foci and thickened walls in the stomachs were seen at 400 mg/kg bw*d and above. Microscopic changes were observed in the stomach, duodenum, salivary glands, and kidney. In the stomach, these changes included basal and squamous cell hyperplasia of the limiting ridge at 400 and 2000 mg/kg bw/d. At 2000 mg/kg bw/d, mild inflammation and foveolor epithelial hyperplasia were seen in the stomach, Brunner's gland hypertrophy was seen in the duodenum, and acinar cell hypertrophy was seen in the salivary gland. Based on the stomach lesions, the NOAEL was 50 mg/kg bw/d, with a LOAEL of 400 mg/kg bw/d.

In a combined oral feed 80-week toxicity and carcinogenicity study in mice, comparable to OECD TG 453, **KHFo** was administered in the diet at 0, 50, 400, and 2,000 mg/kg bw/d. Body weight gain was markedly lower (approx 15%) in the males at the highest dose. At 2000 mg/kg bw/d, mucosal hyperplasia was seen in the stomachs, which was characterized by a minor increase of thickness and folding of the squamous epithelium of the limiting ridge in males, but not in females. Based on these stomach lesions, the NOAEL was 400 mg/kg bw/d and the LOAEL was 2000 mg/kg bw/d.

Conclusion: Inhalation data for FoA and oral (dietary) toxicity data for KHFo are used as read across in this assessment for the other category members. The NOAECs for inhalation studies

with FoA range from 0.062 mg/L/day (mice) to 0.122 mg/L/day (rats) with respiratory tract irritation as the main effect. The three dietary studies of KHFo showed a range for NOAELs of 50 (rat) to 400 (mice) mg/kg-bw/day and a LOAEL range from 400 (rat) to 2000 (mice) mg/kg-bw/day with main effects on body weights and stomach.

Genotoxicity – Gene Mutations

Ames tests (OECD TG 471) performed on **FoA** (and NaFo, as this is formed whenever FoA is tested in buffers containing Na), **CaFo** and **KHFo**, with and without metabolic activation did not induce gene mutations. FoA was also negative in the HGPRT forward mutation test (OECD TG 476) using Chinese hamster ovary (CHO) cells at concentrations up to 500 µg/mL. In an *in vivo* sex-linked recessive lethal test in *Drosophila melanogaster* (similar to OECD TG 477), 0.1% FoA vapor resulted in mutations that were statistically significant. FoA in feed increased mutation frequency (but without statistical significance), and NaFo (in feed neutralized FoA with NaOH) did not induce gene mutations. **KHFo** was negative for gene mutations in an *in vitro* assay using mouse lymphoma L5178 cells (OECD TG 476) at test concentrations up to and including 1200 µg/mL.

Genotoxicity – Chromosomal Aberrations

In an in vitro chromosomal aberration test (OECD TG 473) using Chinese hamster ovary (CHO) cells, **FoA** induced chromosomal aberrations at concentrations of 10-14 mM (associated with pHs of 6.0 to 6.8) but not at lower concentrations associated with higher pH values. Also, when tested using a buffer, FoA did not induce chromosome aberrations unless the buffer capacity was exceeded (e.g., at FoA concentrations of 25-27.5 mM and pH values of 5.7-6.7).

KHFo was negative for chromosomal aberrations when tested in an *in vitro* assay using human peripheral blood lymphocytes (OECD TG 473) and did not increase percent of micronuclei in an *in vivo* rat bone marrow micronucleus test performed according to an European Economic Community (EEC) Directive (similar to OECD TG 474) when tested at up to 50 mg/kg bw.

Genotoxicity – DNA Effects

FoA did not induce sister chromatid exchange (SCE) in Chinese hamster V79 cells (OECD TG 476) or in human lymphocytes at concentrations lower than 10 mM. However, SCEs were induced by FoA at 10 mM in human lymphocytes.

FoA and its salts were not gene mutagens in bacterial or mammalian cells *in vitro*. There is evidence that FoA may be a chromosome mutagen in mammalian cells *in vitro*, but, due to issues of pH and high dosage levels, the evidence is equivocal. There are data on KHFo that indicate that FoA and its salts were not mutagenic *in vivo* in mammals.

Conclusion: Genetic toxicity data (gene mutation and chromosomal aberrations) for FoA, NaFo and KHFo were negative based on weight of evidence evaluation and can be extrapolated to the other category members.

Carcinogenicity

In oral feed studies comparable to OECD TG 453, **KHFo** did not show potential for carcinogenicity in mice in an 80-week study or in rats in a 104-week test using doses up to 2,000 mg/kg bw/d. It is unlikely that the other category members would have the potential to exhibit carcinogenicity.

Conclusion: KHFo did not show potential for carcinogenicity in two long-term rodent feed studies. This can be extrapolated to FoA and the other salts.

Reproductive Toxicity

Reproductive organs were examined in rats and mice exposed to **FoA** at 0, 8, 16, 32, 64, and 128 ppm (0, 0.015, 0.030, 0.061, 0.122, and 0.244 mg/(L*d) in the 13-week studies described above (OECD TG 413). In rats, there were no effects on testicular or epididymal weights, sperm density and sperm motility, or estrous cycles. In mice, sperm motility values were lower at all concentrations, but no dose-response relationship was seen and the values were within the range of historical controls. There were no effects in female mice. The NOAEC was therefore 0.244 mg/L,

the highest concentration used.

Conclusion: No reproductive toxicity data were available on the category members but evaluation of reproductive organs from the repeated-dose toxicity studies in rats and mice showed no effects on reproductive organs. This data can be extrapolated to the other category members.

Developmental Toxicity

In a developmental study, female rats (25/dose, OECD TG 414) were given **NaFo** via oral gavage at 0, 59, 236, and 945 mg/kg bw/d during gestation days 6 to 19. Maternal toxicity was not seen and there were no effects on the developing fetuses. No malformations or skeletal variations were seen. The NOAEL for maternal and developmental toxicity was 945 mg/kg bw/d, the highest dose tested.

In a developmental study (OECD TG 414), rabbits were administered NaFo at 0, 100, 300, and 1000 mg/kg bw/d via oral gavage during gestation days 6 to 28. The following parameters were increased without statistical significance and were in the range of the historical control data (2003-2006) of the same rabbit strain and laboratory: The post-implantation losses of 13.0 and 13.9% at 300 and 1000 mg/kg bw/d, respectively compared with 7.3% in controls (range of historical control: 5.8 – 50%); the total external, skeletal, and soft tissue malformations were 6.7% at 1000 mg/kg bw/d compared with 3.8% in controls (range of historical control 1.1 – 8.7 %). The incidence of total variations (external, skeletal, and soft tissue) was 66.1 to 67.2% in treatment groups compared with 58.0% in controls (range of historical control 55.9 – 85.1%). The NOAEL for maternal toxicity and prenatal developmental toxicity was determined to be 1000 mg/kg bw/d, the highest dose tested.

The effect of **KHFo** on breeding sows was examined in 27 female pigs exposed to 0, 1.2, and 3.6% KHFo in feed (approximately 0, 140, and 430 mg/kg bw/d; group sizes of 7 – 8 pigs) at day 28 before the first mating and extended through two breeding periods until weaning of the second breed. Total exposure period was > 300 days. The study design was not comparable to an OECD method. The NOAEL for maternal toxicity and reproductive effects was 430 mg/kg bw/d, the highest dose tested.

In a non-guideline reproductive/developmental study, 42 female pigs were mated and administered 0, 1.2, 3, or 6 % KHFo in feed (equivalent to 0, 157, 384, or 753 mg/kg bw/d during gestation. The sows received the test diets for more than 150 days, from acquisition through gestation and lactation until weaning. No maternal, reproductive, or developmental toxicity was seen and the NOAEL was determined to be 753 mg/kg bw/d (the highest dose tested).

Supporting information is available from several non-guideline studies. No toxicity for reproduction and development was seen in two multi-generation studies using Wistar rats receiving **CaFo** at 0.2% in drinking water (approx. 150-200 mg/kg bw/d) in one study and 0.4% (approx. 300-400 mg/kg bw/d) in a second study, although details from these studies were limited.

Conclusion: Developmental toxicity studies for NaFo in rats and rabbits showed no effects on the developing fetuses with NOAEL values of 945 and 1000 mg/kg-bw/day, respectively. These data can be extrapolated to the other category members.

Subcategory II: Methyl Formate

Acute Toxicity

A 4-hr **inhalation** LC_{50} of 35 mg/L was obtained for MeFo in an OECD TG 403 study with rats. At the lowest dose (25 mg/L), animals exhibited gasping. Additional signs at higher concentrations included poor coordination, prostration, lacrimation, salivation, lung congestion with some scattered hemorrhages and fluid. In other acute inhalation studies using MeFo, animals exhibited forced breathing and heart dilatation and myodegeneration. Mortality was 100% within minutes when rats were exposed to saturated atmospheres of either FoA or MeFo.

In two separate studies for MeFo, **oral** LD_{50} values in rats were 1382 and 1500 mg/kg bw (similar to OECD TG 401). Animals showed signs of sluggish, heavy breathing/gasping, hemorrhages in the lungs, and discolored/mottled livers, spleens, kidneys, stomachs and intestines. Other signs included

difficulty breathing, apathy, unsteady gait, and corrosion of intestines or gastritis in the stomach.

The acute dermal LD_{50} of **MeFo** in rabbits was > 15,680 mg/kg bw with no clinical signs reported (study similar to OECD TG 402).

Conclusion: Acute toxicity of MeFo is low via inhalation and oral routes.

Irritation/Sensitisation

MeFo was slightly irritating under 24-hour occlusive skin contact (similar to OECD TG 404 except for prolonged occlusive skin contact). **MeFo** was irritating to the eyes of rabbits (method according to US Federal Register guidelines). Sensitization data on **MeFo** were not located. **MeFo** showed signs consistent with respiratory tract irritation in acute inhalation toxicity studies (OECD TG 403).

Conclusion: Acute toxicity of MeFo was low via inhalation and moderate via oral route. Methyl formate was slightly irritating to rabbit skin and irritating to rabbit eyes. MeFo showed signs of respiratory tract irritation in an acute inhalation toxicity study. Sensitization data on MeFo were not located.

Repeated-Dose Toxicity

Methyl Formate

Repeated dose toxicity data on MeFo were restricted to an inhalation study where MeFo was administered as a vapor to Wistar rats at concentrations of 0, 100, 500, or 1500 ppm (0, 0.252, 1.237, or 3.693 mg/(L*d)) for 2 weeks, 6 h/day, 5 days/week (OECD TG 412). Terminal body weights were markedly decreased in both sexes at the highest concentration. Changes in several organ weights were observed (liver, lung, kidneys, and spleen) at the highest concentration. In addition, histopathological changes in the nasal epithelium, squamoid metaplasia, and infiltration of inflammatory cells into the respiratory tract were observed at the middle and highest concentration in a dose-related manner. Based on respiratory tract changes and effects on body weight and organ weights, the NOAEC and LOAEC for local and systemic effects were 0.252 mg/(L*d) and 1.237 mg/(L*d), respectively.

Methanol (Supporting chemical)

Data were available for the supporting chemical methanol from 20-day and 29-month inhalation studies in monkeys, 12-month studies in rats and mice and a 13-week gavage test in rats. No dermal studies were available. It should be noted that NOAEC/NOAEL values from rodent studies must be interpreted with caution because of the rapid metabolism of formate to CO_2 in rodents compared to humans.

In a whole body inhalation study in monkeys exposed to 0, 0.013, 0.13, and 1.3 mg/L methanol was given for 21 hours/day, 7 days/week for 7, 19, and 29 months. Several general clinical signs as well as degenerative effects in the brain (at 0.13 and 1.3 mg/(L*d)), slight peripheral nerve damage (at 0.13 and 1.3 mg/(L*d)), very slight degeneration of the optic nerve (concentrations not noted), increased fat granules and slight fibrosis in the liver (all concentrations), and Sudan positive granules in the kidney (at 0.13 and 1.3 mg/(L*d)) were observed. Also, a slight myocardial disorder (at 0.13 and 1.3 mg/(L*d)), localized effects in the trachea and possible slight fibrosis in the lungs (concentrations not noted) were observed. Although the statistical significance of the effects cannot be verified from the study report, the number of effects and systems affected indicate a relationship with methanol.

In another whole body inhalation study in monkeys exposed up to 20 days for 21 hours/day, coma and lethality were observed at concentrations > 9.1 mg methanol/(L*d). At 6.5 mg/(L*d), necrosis of the basal ganglia plus cerebral edema were observed in the brain and fibrosis was seen in the liver. Partly vacuolated hyaline degeneration in the kidney was also seen at this concentration. At 3.9 mg/(L*d), hyperplasia and fibrosis around myelin sheaths of the basal ganglia, increases in astroglia cells and mild fatty liver were observed. The optic nerve showed atrophy at 3.9 mg/(L*d) and above, along with reduction in myelin fibers.

In a whole body inhalation study in mice exposed for 12 months to concentrations of 0, 0.013, 0.13, and 1.3 mg methanol/(L*d) 20 hours/day, slight changes in clinical signs, body and organ weights, and some changes in histopathology were observed. In rats exposed in the same manner, slight changes in body weight and organ weights were observed at the highest concentration. The NOEC was 0.13 mg/(L*d). In rats, gavage doses of 100, 500, and 2,500 mg/kg bw/d for 90 days resulted in increased liver enzymes and reduced brain weights at the highest dose resulting in determining a NOAEL of 500 mg methanol/kg bw/d.

Conclusion: The NOAEC for inhalation studies with MeFo was 0.252 mg/L/day (rat) with a LOAEC of 1.237 mg/L/day based on respiratory tract irritation as the main effect.

Genotoxicity – Gene Mutations

Methyl Formate

MeFo did not induce gene mutations in an Ames test (OECD TG 471), with and without metabolic activation.

Methanol (Supporting chemical)

Data on methanol (supporting substance) were used for the chromosomal aberrations endpoint. Of four *in vitro* micronucleus and cytogenetic assays and ten *in vivo* micronucleus and cytogenicity assays, all were negative for chromosomal aberrations except one cytogenetic assay, which was positive for aneuploidy, sister chromatid exchange, and micronuclei. Thus, most studies indicate that methanol does not have the potential to induce chromosomal aberrations.

Conclusion: MeFo did not induce gene mutations in bacteria. Weight of evidence evaluation indicates that methanol (supporting substance) does not have the potential to induce chromosomal aberrations. MeFo is therefore unlikely to be genotoxic.

Carcinogenicity

Methyl Formate

No data.

Methanol (Supporting chemical)

There was no evidence of a carcinogenic potential when methanol was tested in two valid long-term whole body inhalation studies in rats (24 months) and mice (18 months) at exposure concentrations up to 1.3 mg/L, 19 or 20 hours per day.

Conclusion: MeFo is unlikely to have a potential for carcinogenicity, based on the negative results obtained for methanol.

Reproductive Toxicity

Methyl Formate

No data.

Methanol (Supporting chemical)

Reproductive and developmental toxicity studies were available for **methanol** (supporting substance for methyl formate) in monkeys, rats, and mice. Methanol was evaluated in the OECD SIDS program (SIAM 19).

In monkeys, parents were exposed via inhalation prior to and during breeding as well as during pregnancy to concentrations of 0, 0.26, 0.78, and 2.34 mg/(L*d). A late wasting syndrome was observed at the highest dose in 2/7 female offspring, with signs of severe malnutrition and gastroenteritis. Mild neurobehavioral effects in offspring and some vaginal bleeding in mothers were seen at all concentrations; an association with the test substance was difficult to establish.

Several inhalation studies in rats resulted in a variety of effects in offspring due to prenatal and/or postnatal dosing. In a 2-generation whole body inhalation reproductive study in which rats were exposed for 19-20 hours/day, decreased brain weights in the first and second generation offspring (F1, F2) resulted in a NOAEC of 0.13 mg/(L*d).

In a study of reproductive effects in mice, there were no treatment-related effects after oral dosing up to 1000 mg/kg bw/d for five weeks.

Developmental Toxicity

Methyl Formate

No data.

Methanol (Supporting chemical)

In a developmental study rats were exposed by whole body inhalation on gestation days 1 to 19 at the two lowest concentrations of 6.63 mg/L and 13.3 mg/L and on days 7 to 15 at the highest concentration of 26.6 mg/L for 7 hours/day. Malformations and fetal weight changes resulted in a NOAEC of 6.5 mg/(L*d). In a second whole-body rat inhalation developmental study (gestation days 7 to 17 for 23 hours/day), malformations, increased fetal resorptions, and fewer live fetuses were observed, resulting in a NOAEC of 1.3 mg/(L*d).

A developmental whole body inhalation study in mice exposed on gestation days 6 to 15 for 7 hrs/day resulted in increased exencephaly and cleft palate, fully resorbed fetuses, decreased numbers of live pups, and decreased body weights, with a NOAEC of 1.3 mg/(L*d). Oral studies in mice resulted in malformations at 4000 mg/kg bw/d (the LOAEL) and higher; no NOAELs could be established from these studies.

Blood methanol concentrations associated with serious teratogenic effects and reproductive toxicity observed in the rodent studies were in the range of 1000 – 2000 mg methanol/L blood, which is associated with formate accumulation in humans, and can result in metabolic acidosis and clinical signs. Also humans have exhibited transient central nervous system (CNS) effects at blood methanol levels above 200 mg/L and impairment of vision at levels above 500 mg/L. Fatalities have occurred in untreated patients with initial methanol concentrations in the range of 1500-2000 mg/L.

Conclusion: In the absence of data on MeFo, read across of the data on the salts and methanol was used. Via the oral route, the NOAEL of NaFo was 945 mg/kg bw/day in rat and rabbit developmental toxicity studies, and the LOAEL for methanol was 4000 mg/kg bw/day in oral studies using mice.

The inhalation NOAEC for methanol was 1.3 mg/(L*d) in studies using rats and mice. A conservative NOAEC for MeFo can be estimated to be 2.44 mg/(L*d) if one assumes that all the methyl formate is hydrolysed to methanol (one mole of MeFo gives one mole of formate and one mole of methanol), and that the toxicity of MeFo is dominated by methanol.

NTP concluded that rodent data on reproductive and developmental toxicity of methanol are relevant for humans despite the known differences in methanol metabolism between rodents and humans. Rodents are adequate models for human exposure to methanol at levels where formate does not accumulate. However, blood methanol concentrations associated with serious teratogenic effects and reproductive toxicity are in the range associated with formate accumulation, which is likely to result in metabolic acidosis, and visual and clinical effects in humans. Other effects (e.g., subtle, not yet definitive neurological effects observed in primates) may be exhibited at lower inhalation doses and lower methanol blood levels.

ENVIRONMENT

Subcategory I: Formic acid and its dissociative salts

At room temperature **FoA** is a colorless liquid. The **salts NaFo**, **AFo**, **CaFo**, **KFo**, and **KHFo** are white or colorless solids. The melting point of the pure FoA is 4 °C. The salts have melting points

ranging from 108.4 °C (KHFo) to > 300 °C (CaFo). The boiling point for pure FoA is 100.2 °C. The corresponding boiling points of the salts range from > 130 °C to > 350 °C with indication for decomposition. The relative densities of the category members at ambient temperature vary between 1.22 (FoA, 20 °C, liquid) and 2.015 (CaFo, solid). The category members are all miscible in water with solubility ranging from 144 g/L (CaFo) to 972 g/L (NaFo). The pure FoA is semi-volatile with a vapor pressure of 42.7 hPa at 20 °C. The Henry's Law Constant (HLC) for pure FoA is 0.014 $Pa*m^3/mol$ at 20 °C. The salts have low to negligible vapor pressures and HLC values, which will be enhanced in aqueous solution due to dissociation to formate and the counter ions. The low log K_{ow} values of < 0 and the calculated BCF values of 3.2 show low potential for bioaccumulation for all category members. The dissociation constant (pKa) of pure FoA was experimentally determined to be 3.70 at 20 °C. Compared to the pure substance, formic acid in aqueous solution shows some different physico-chemical properties. For an 85% solution, the melting point is -50.8 °C, the boiling point is 107.3 °C at 1013 hPa and the vapor pressure at 20 °C is 24.2 hPa.

In the atmosphere, **FoA** will be photodegraded by reactions with OH radicals with a half-life of 36 days. **FoA** will not undergo hydrolysis at pH 4, 7, or 9. The same refers to the salts, where the formate anion and the corresponding cations are hydrolytically stable.

The category members can generally be regarded as **readily biodegradable** as demonstrated in ready biodegradability tests. In two Modified OECD Screening Tests following OECD TG 301E, **FoA** was degraded to 99 and 98 % related to DOC after 11 and 14 days, respectively. More than 75 % of **CaFo** (BOD/ThOD) was degraded after 20 days in a Closed Bottle Test following OECD TG 301D, and 70.5 % of **AFo** (BOD/ThOD) was degraded after 28 days in a Manometric Respirometry Test performed according to OECD TG 301F. Following OECD TG 301D, the biodegradation rate of **KFo** was 82 and 92 % (BOD/ThOD), respectively, each after 28 days.

According to Mackay Level I, the category members are likely to distribute nearly completely into water. Only small amounts of **FoA** (6.5 %) would also distribute to air. Using Mackay Level III, emissions of FoA to air (33 %), water (33 %), and soil (33 %) would lead to equal distributions of ca. 45 % each into water and soil. The next largest amount would be found in air (8 %).

For most category members reliable studies on acute toxicity to fish, *Daphnia* and algae growth inhibition were available.
Tests using **FoA** show EC/LC_{50} values between 1 and 100 mg/L. These results appear to be due to acidity as demonstrated in the test with *Leuciscus idus*, where a neutralized test solution of 100 mg/L produced no mortality. For the **salts** experimental acute EC/LC_{50} values were > 100 mg/L.
In a chronic toxicity test following OECD TG 211, Daphnia magna was given **FoA** under neutralized conditions; the 21-d NOEC for effects on reproduction was 100 mg/L.

Subcategory II: Methyl Formate

MeFo is a colorless liquid at room temperature. The melting point is at -99.8 °C. The boiling point for pure MeFo is 31.5 °C and the relative density at 20 °C is 0.97. MeFo is volatile with a vapor pressure of 781 hPa (25 °C). It has a HLC of 22.6 $Pa*m^3/mol$ at 25 °C. The log K_{ow} value was measured to be 0.03 and the BCF was correspondingly calculated to be 3.2.

In the atmosphere, **MeFo** will be photodegraded by reactions with OH radicals with a half-life of 67 days. **MeFo** was shown to have a hydrolysis half-life of 67 hours at pH 7.0 from an experimentally-derived alkaline hydrolysis rate. **MeFo** was found to be **readily** biodegradable with 93 % degradation after 28 days (TIC/ThIC) in the CO_2-Headspace Test following OECD TG 310.

According to Mackay Level I and due to its volatility, **MeFo** would distribute nearly completely to air (98.1 %). Using Mackay Level III, emissions of MeFo to air (33 %), water (33 %), and soil (33 %) would lead to equal distributions of ca. 45 % each into air and water. The next largest amount would be found in soil (10 %).

For aquatic toxicity, **MeFo** was tested in 96-hour static study with the golden orfe (*Leuciscus idus*), the 96-h LC50 was calculated to be approximately 115 mg/L. The 48-h EC50 for *Daphnia magna* was > 500 mg/L, based on nominal concentrations. Green algae, *Desmodesmus subspicatus*, were exposed to MeFo over 96 hours to nominal concentration, the 72-h EbC50 and ErC50 were 351 and

1063 mg/L, respectively. **MeFo** showed the experimental acute toxicity of EC/LC_{50} values > 100 mg/L.

EXPOSURE

Subcategory I: Formic acid and its dissociative salts

The annual world production capacity of **FoA** in 2006 was estimated at 450,000 – 600,000 metric tons. Most of the producers are located in the Asia/Pacific region (more than 10 different production sites). Additional sites are in Western Europe (3 sites) and North/South America (1 site).

The total global capacities of the **formates** were estimated at 100,000 – 150,000 (NaFo), 10,000 – 25,000 (each AFo and KHFo), 25,000 – 50,000 (KFo) and 50,000 – 100,000 (CaFo) metric tons. The capacities for CaFo can be subdivided into > 85 % for Europe, approximately 10 % for Asia/Pacific, and < 5 % for North/South America. Most of the big producers are located in Europe (more than 7 different production sites).

Several of the category members are used to manufacture other category members or are byproducts of a manufacturing process. **FoA** is mainly produced by hydrolysis of MeFo but can also be produced using NaFo. **NaFo** and **CaFo** are byproducts in the synthesis of polyols such as pentaerythritol and can be produced directly from the corresponding hydroxide and carbon monoxide. **AFo** can be prepared from FoA and ammonia and can be produced using CaFo. **KHFo** is produced from FoA and KFo.

FoA is used as acidulant and decalcifying agent, for pH-adjustment in cosmetic formulations as well as in dye baths for dyeing of natural and synthetic fibers. Other applications of FoA are as an additive for cleaning agents, in the synthesis of the sweetener aspartame, and in the desulphurization of flue gas. In the EU, FoA has been notified for the Biocidal Products Directive (98/8/EC) and complete dossiers have been submitted in 2007/2008 for registration. A total of 422 preparations containing FoA were registered in 2003 in the Nordic countries Denmark, Finland, Norway, and Sweden. The main amount of FoA (by weight) was used in the categories "food/feedstuff flavourings and nutrients," "surface treatment," "pH-regulation agents," and "non-agricultural pesticides and preservatives." The largest number of FoA-containing preparations was in the categories "cleaning/washing agents" (> 100 listings) and "adhesives, binding agents" (> 40 listings).

NaFo is used to produce FoA. Also, an important process for manufacturing sodium dithionite starts with NaFo. Oxalic acid production employs NaFo as an intermediate. NaFo is also used in chrome tanning and as a mordant in the dyeing and printing of fabrics by the textile industry. The reducing power of NaFo is used in electroplating baths and photographic fixing baths. In chemistry applications, it is employed as precipitant for noble metals, for buffering of strong mineral acids to higher pH and as reducing, complexing, and analytical agents. In medication, it is applied as a caustic, astringent agent, and in foodstuffs as additive for preservation. Other usages for NaFo are as runway deicer, drilling and completion fluids, and enzyme stabilizer in liquid detergents. NaFo is also used as a processing chemical in the manufacturing sector and in the oil/gas exploration industry. In 2003, 239 preparations containing NaFo were registered in the Nordic countries adding up to a total annual volume of about 23,417 metric tons of NaFo.

AFo solutions are used as a low corrosion silage aid. In chemistry applications, AFo is employed for separation of base from noble metals, for production of amines, and as a buffer. A total of 15 preparations containing AFo were registered in the Nordic countries adding up to a total annual volume of about 74 metric tons of AFo.

Major uses of **CaFo** are as concrete admixture, animal feed additive and as chemical intermediate. CaFo is further used in tanning (leather industry) and as preservative for silage and food. It is applied as a binder for fine ore briquets, in drilling fluids and lubricants, for flue gas scrubbing and as concrete settings accelerators. CaFo is also used for the production of FoA. In 2003, 150 preparations containing CaFo were registered in the Nordic countries and were mostly categorized as

"adhesives/binding agents", "construction materials", "fillers", and "process regulators".

Main usages of **KFo** are as drilling and completion fluids and runway deicer. Moreover, KFo is applied as a secondary coolant for indirect cooling systems, in fire extinguishing applications, in wood preservation applications and as insulation material. KFo is further applied in fungicides and bactericides for agriculture, in chemical synthesis, e.g. to prepare potassium oxalate, as a catalyst/process regulator and as a polyurethane component. In 2003, 24 preparations containing KFo were registered in the Nordic countries adding up to a total annual volume of about 1,361 metric tons of KFo.

KHFo is used in feed mixtures for pigs as a growth promoter. KHFo is also applied as an intermediate in chemical synthesis, in food/foodstuff additives, and in pharmaceuticals.

Subcategory II: Methyl Formate

The annual world production capacity in 2005 for **MeFo** was estimated at 500,000 – 700,000 metric tons. The major producers are located in the European Union (2 different productions sites). Additional sites are located in the Asia/Pacific region (5-8 sites) and USA (1 site). **MeFo** is produced by base-catalyzed carbonylation of methanol, but can also be produced by oxy-dehydrogenation of methanol or by heating methanol with NaFo and hydrochloric acid with subsequent distillation.

Most of the **MeFo** produced is used as an intermediate in the production of FoA and formamide. It can also be used as a starting material in the production of high purity carbon monoxide. Dimethylformamide is produced by reacting MeFo with dimethylamine. A new use has been found for MeFo in the production of foundry molds. MeFo is also employed as a solvent for fats, oils, fatty acids, cellulose ester and acrylic resins. It has crop protecting applications as fumigant and larvicide for tobacco and food crops. Use as high-boiling refrigerant for house appliances is also reported. In 1999 - 2001, four MeFo-containing preparations were registered in Denmark.

Environmental exposure

Subcategory I: Formic acid and its dissociative salts

The occurrence of **formates** in the environment is ubiquitous. In food, **FoA** occurs naturally in animals, plants and foods such as fruits (20 – 40 mg/kg), honey (20 – 2000 mg/kg), wine (1 – 340 mg/kg), roasted coffee (30 – 40 mg/kg), evaporated milk (30 – 40 mg/kg), and cheese (20 – 200 mg/kg). FoA is also added intentionally to some foods as a flavor adjunct.

In the atmosphere, photochemical reactions, i.e. ozone-olefin reaction, isoprene oxidation, gas-phase reaction of formaldehyde with $HO_2\bullet$, and aqueous phase oxidation of formaldehyde, are important indirect sources of FoA formation. In mid-latitude continental regions, possible sources of FoA are direct emission from vegetation and biomass burning. In tropical continental sites, direct emission from vehicles, ants, soil, vegetation, and biomass-burning are important sources for FoA.

Newer findings in tropical forest studies suggest that the exchange of FoA and acetic acid between vegetation and the atmosphere is due to a bidirectional exchange behavior of the plants. The tropical forest is a sink rather than a source for organic acids. High atmospheric concentrations of organic acids in Brazil during the dry season have been attributed to biomass burning. During the wet season (low biomass burning activity), indirect emission by the vegetation, i.e. photochemical oxidation of primarily emitted biogenic hydrocarbons, was assumed to dominantly contribute to the atmospheric burden of the organic acids. FoA is scavenged by wet and dry deposition with wet deposition being more effective than dry deposition.

The possible sources at marine locations are photochemical reactions, biogenic emissions, and long-range transport.

Concentrations of FoA, as detected in the atmosphere, range from below the detection limit to about

35 µg/m³ depending on season and location (urban/suburban, rural/remote, lower/upper troposphere, etc.). FoA concentrations in residential houses in suburban and urban areas ranged from > 16 µg/m³ up to ca. 35 µg/m³. Mean total concentrations of FoA motor exhaust emissions were determined to be 29 - 106 µg/m³ (gasoline) and 225 µg/m³ (diesel). When typical biomass fuels like wood and bushes with or without leaves are burned, FoA can reach smoke concentrations of up to 1 mg/m³. Emitted amounts of FoA from painted steel ranged from 0.4 to 30 mg/m³.

According to the data reported to the German Emission Register 2004, during production and processing 11,660 kg of FoA were emitted to air at a production and processing site in Germany in 2004. One company in Finland and the Netherlands reported that during production and processing, AFo and KFo were not emitted to air or water.

Because of their high solubility, formic and acetic acids are the most abundant acids in rainwater and are significant in the acidification of rainwater. Concentrations of FoA in rainwater around the world range from 55.2 to 340.4 µg/L at marine sites, from below the detection limit to 1048.8 µg/L at remote sites, from 354.2 to 1035 µg/L at semi-urban sites, and from 36.8 to 492.2 µg/L at urban sites. Clouds are considered important for FoA, providing either a source or sink for it. Average concentrations of FoA in fog, dew and cloud water were reported to be 0.2 – 2.3 mg/L (max. 29 mg/L). Surface water concentrations between 12 and 115 µg/L were determined. FoA concentration in the 0 – 1 cm layer of sediments ranged from 23 to 437 mg/kg wet weight. Depending on the industry, FoA concentrations of up to 584 mg/L were reported from effluents before waste water treatment (produced water from oil production platforms).

Subcategory II: Methyl Formate

MeFo production and use as a solvent and organic synthesis intermediate may result in its release to the environment through various waste streams. MeFo use as a fumigant and larvicide will result in its direct release to the environment. MeFo was detected in the volatiles of chicken, beef, and pork flavor. MeFo was identified as a volatile constituent in brewed, roasted, and dried coffee. It has also been detected as an aroma substance in apples. MeFo was qualitatively identified in the volatile emissions of leaves litter from poplar trees, in drinking water samples, and in vapor-phase ambient air samples.

MeFo was also detected in cigarette smoke and gasoline engine exhaust. It is present in wastewater from urea-formaldehyde resin manufacturing plants. MeFo is released in vent effluents during the commercial production of methanol. It can be released in waste streams from its commercial manufacture and use in the production of formamide. MeFo has also been detected in exhaust gases from the combustion of various hydrocarbon fuels (< 0.1 to 0.7 ppm).

According to the data reported to the German Emission Register 2004, during production and processing 3,150 kg of MeFo were emitted to air at a production and processing site in Germany in 2004.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health: The chemicals of the formic acid and dissociative salts subcategory are currently of low priority for further work. The chemicals possess properties indicating a hazard for human health (moderate inhalation toxicity of FoA; local effects including corrosion of skin (FoA) and eye (FoA, KHFo), eye irritation (NaFo, CaFo), respiratory tract irritation (FoA, KHFo), and stomach irritation following repeated high doses (KHFo)). Exposure in occupational settings is controlled. Countries may wish to investigate any exposure scenarios that have not been presented by the Sponsor country.

MeFo is a candidate for further work based on ongoing work related to methanol. MeFo and

methanol (its metabolic product) possesses properties indicating a hazard for human health (MeFo: moderate inhalation toxicity, eye and respiratory tract irritation; methanol: neurological effects, CNS depression, ocular effects, and reproductive/developmental toxicity). In the US, further work is being performed on methanol regarding the use and refinement of pharmacokinetic models for extrapolating animal data to humans.

Environment: The chemicals of this category are currently of low priority for further work due to their low hazard profiles. Formic acid has properties indicating a hazard for the environment (acute toxicity to aquatic organisms between 1 and 100 mg/l) due to pH effects. This chemical, however, is also of low priority for further work for the environment because of its rapid biodegradation and limited potential for bioaccumulation.