

Test Plan for Methyl 4-formylbenzoate

Methyl 4-formylbenzoate

CAS No. 001571-08-0

Eastman Chemical Company

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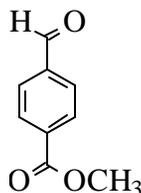
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The High Production Volume Challenge (HPVC) Program

Test Plan for

Methyl 4-formylbenzoate

1 IDENTITY OF SUBSTANCES



Methyl 4-formylbenzoate

CAS NO. 001571-08-0

Synonyms:

4-formyl benzoic acid, methyl ester
terephthalaldehydic acid, methyl ester
methyl terephthalaldehydate
p-formylbenzoic acid, methyl ester
4-(methoxycarbonyl) benzaldehyde
4-carbomethoxybenzaldehyde
methylbenzaldehyde-4-carboxylate
4-carboxybenzaldehyde, methyl ester
p-carbomethoxybenzaldehyde

2 CHEMICAL ANALYSIS

2.1 INTRODUCTION

In November of 1999, Eastman Chemical Company (Eastman) committed to participate in the Chemical “Right-to-Know” Program. As part of this commitment, Eastman is committed to assembling and reviewing available test data, developing and providing test plans for methyl 4-formylbenzoate, and, where needed, conducting additional testing. The test plan and robust summaries presented are the first phase of Eastman’s commitment to the Chemical “Right-to-Know” Program.

2.2 BACKGROUND INFORMATION

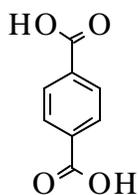
Methyl 4-formylbenzoate is a by-product in the manufacture of dimethyl terephthalate. Methyl 4-formylbenzoate is separated from the dimethyl terephthalate by distillation. The composition of the isolated methyl 4-formylbenzoate is greater than 90%. Other minor secondary components include less than 3% dimethyl terephthalate, less than 2% methyl 4-carboxybenzoate, less than 2% methyl 4-methylbenzoic acid, and smaller amounts of benzoic acid and 4-methylbenzoic acid.

As a by-product in the preparation of dimethyl terephthalate, methyl 4-formylbenzoate has limited commercial use. It is mixed with a number of other chemicals to make blends used in sand castings. The blend material is completely oxidized either in the casting process or in the treatment of the sand. The castings are used predominantly in the USA automotive market. With its only use being in industrial applications there is minimal opportunity for exposure to the general public and exposures in the workplace are minimized through appropriate industrial hygiene practices.

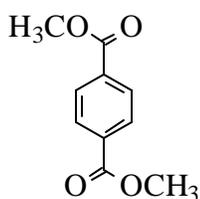
2.3 STRUCTURAL CLASSIFICATION

Methyl 4-formylbenzoate is a benzene derivative ring-substituted with a methyl ester and an aldehyde functional group. The functional groups are situated on the 1- and 4-position of the aromatic nucleus. The mono-substituted aromatic substances methyl benzoate and

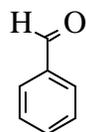
benzaldehyde each contain one of the two functional groups present in methyl 4-formylbenzoate. Given that the aldehyde function is oxidized to a carboxylic acid (see Section 2.4.2) and that the methyl ester is hydrolyzed to yield the corresponding carboxylic acid (see Section 2.4.1), methyl 4-formylbenzoate readily forms the diacid terephthalic acid (*i.e.*, 1,4-benzenedicarboxylic acid; terephthalic acid) in nature and as a metabolite in animals. This test plan includes data for methyl 4-formylbenzoate and several related benzyl derivatives including benzaldehyde, methyl benzoate, sodium benzoate, benzoic acid, terephthalic acid, and dimethyl terephthalate, the latter, like methyl 4-formylbenzoate, also hydrolyzes to terephthalic acid. As stable animal metabolites, benzoic acid and 1,4-benzenedicarboxylic acid are excreted primarily in the urine either free or conjugated with glycine. These reaction pathways have been reported in both aquatic and terrestrial species. Based on the polarity of product formed by ester hydrolysis and aldehyde oxidation (*i.e.*, terephthalic acid), it is concluded that the metabolite is rapidly eliminated and, therefore, exhibits low toxicologic potential.



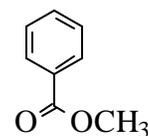
terephthalic acid



dimethyl
terephthalate



benzaldehyde



methyl benzoate

The data summarized in the test plan and data recorded in the robust summaries for structurally related substances have been previously submitted to the Office of Economic and Community Development (OECD) and the U.S. Environmental Protection Agency (EPA). Data for terephthalic acid and dimethyl terephthalate were submitted in the form of Safety Information Data Sheets (SIDS) to the OECD by the EPA. The test plans and robust summaries were accepted with no requests for additional testing. Robust summaries used in the preparation of these SIDS submissions have been included in this document.

In addition, the Flavor and Fragrance High Production Volume Consortia (FFHPVC) has submitted (12/01) a test plan and robust summaries for the chemical category named “Benzyl Derivatives” including data for ten benzyl derivatives. EPA commented (12/02) on this test plan and robust summaries. The robust summaries for methyl benzoate, benzaldehyde, benzoic acid and sodium benzoate for which no revisions or additional information were requested or ones in which requested information has been added are also included in this document.

2.4 CHEMICAL REACTIVITY AND METABOLISM

2.4.1 Hydrolysis of Esters

The hydrolysis of benzoate esters occurs in the environment and in animals. Uncatalyzed hydrolysis of methyl 4-formylbenzoate occurs readily at a slightly basic pH=9. Greater than 50% hydrolysis to yield 4-formylbenzoic acid was observed after 2.4 hours at pH=9, indicating the half-life of the ester is less than 1 day. The estimated half-life at pH=7 is estimated to be approximately 200 days [Hoffman, 2003]. The rates of enzyme-catalyzed hydrolysis reported for benzoate esters in animals are orders of magnitude greater than uncatalyzed rates mentioned above.

In general, the catalytic activity of carboxylesterases or esterases such as the *beta*-esterases hydrolyzes aromatic esters *in vivo* [Heymann, 1980]. These enzymes are found throughout mammalian tissues, but predominately in hepatocytes [Anders, 1989; Heymann, 1980]. The hydrolysis of benzoate esters yields the corresponding benzoic acid derivatives in several *in vitro* experiments.

In a series of hydrolysis experiments with 4 alkyl benzoates (including methyl benzoate) and 2 aryl benzoates, plasma half-lives ($t_{1/2}$) in 80% human blood plasma decreased from 210 minutes for ethyl benzoate to 24 minutes for butyl benzoate and 19 and 15 minutes for phenyl benzoate and benzyl benzoate, respectively [Nielson and Bundgaard, 1987].

Experiments *in vivo* confirm rapid hydrolysis of benzoate esters in animals. Methyl 2-hydroxybenzoate was orally administered to males rats at a dose equivalent to 500 mg/kg

bw of 2-hydroxybenzoic acid [Davison *et al.*, 1961]. Twenty minutes following dosing, plasma levels showed complete hydrolysis of methyl 2-hydroxy benzoate. Similarly, male dogs were given 320 mg/kg bw of the methyl ester in capsules. After one hour, blood samples showed 95% hydrolysis of methyl ester to 2-hydroxybenzoic acid. In humans given 0.42 ml methyl 2-hydroxybenzoate (approximately 500 mg), blood samples showed 79% of the dose hydrolyzed within the first 90 minutes.

Several experiments were conducted [Jones *et al.*, 1956] to study the hydrolysis of *p*-hydroxybenzoic acid esters. Comparisons were made between oral (1,000 mg/kg bw) and intravenous (50 mg/kg bw) administration in dogs. Methyl and ethyl esters were rapidly hydrolyzed by esterases in the liver and kidney. Recovery of the dose of butyl *p*-hydroxybenzoate was 48% and 40% from the oral and intravenous administration, respectively. Liver preparations from dogs injected with 100 mg/kg bw of the methyl, ethyl, or propyl benzoate showed 100% hydrolysis in 3 minutes; whereas, 100% hydrolysis of the butyl ester occurred after 30-60 minutes.

Carboxylesterase (Type B) activity has been reported in a variety of fish species at different life stages [Leinweber, 1987; Boone and Chambers, 1996; Abas and Hayton, 1997; Barron *et al.*, 1999]. Enzyme activity of rainbow trout sera, liver and whole body homogenates were similar to those of rat liver homogenate. A significant increase (300%) in activity occurred between yolk-sac and juvenile stage of rainbow trout development. Carboxylesterase activity was not significantly different for whole body homogenates of the rainbow trout, channel catfish, fathead minnows, and bluegill [Barron *et al.*, 1999]. These data support the conclusion that simple aromatic benzoate esters are readily hydrolyzed in these animals.

In summary, the methyl 4-formylbenzoate is expected to hydrolyze to the corresponding benzoic acid derivative, 4-formylbenzoic acid, *in vivo*. Complete hydrolysis is expected to occur in gastric juice, intestinal fluid, portal blood and liver. Slower hydrolysis is expected in uncatalyzed environments.

2.4.2 Oxidation of the Aldehyde in Methyl 4-formylbenzoic Acid

The aldehyde functional group in methyl 4-formylbenzoic acid is expected to be readily oxidized by aldehyde dehydrogenase (ALD) to the corresponding benzoic acid derivative. Based on the data that the ester function of methyl 4-formylbenzoate is hydrolyzed prior to absorption, the rapid oxidation of the aldehyde (formyl) function would then yield 1,4-benzenedicarboxylic acid (terephthalic acid) as the principal metabolite of methyl 4-formylbenzoate. Based on the metabolism of related aromatic esters (*i.e.*, benzyl acetate), hydrolysis and oxidation to the benzoic acid derivative is extremely rapid *in vivo*.

When benzyl acetate was fed or administered by gavage to rats, no benzyl acetate, benzyl alcohol or benzaldehyde was observed in the plasma. However, high plasma levels of hippuric acid (glycine conjugate of benzoic acid) and unconjugated benzoic acid were detected indicating that benzyl acetate is rapidly hydrolyzed to benzyl alcohol, which is then rapidly oxidized first to benzaldehyde and then to benzoic acid [Yuan *et al.*, 1995].

Groups of male F344 rats and B6C3F1 mice were administered [¹⁴C]-benzyl acetate orally at levels up to 500 and 1,000 mg/kg bw, respectively, 5 days/week for a period of two weeks [Abdo *et al.*, 1985]. The ester was readily absorbed from the gastrointestinal tract of both species and approximately 90% and 0.3-1.3% of the total dose was recovered as hippuric acid in the urine and feces, respectively, within 24 hours. No benzyl acetate-derived radioactivity was detected in any tissue (*i.e.*, blood, liver, muscle, adipose, skin, lung, kidney and stomach) analyzed at 24 hours. The clearance pattern was not affected at any dose tested. Such complete clearance indicates that aromatic esters are readily absorbed, hydrolyzed to component acids and alcohols, which in turn are oxidized to the corresponding aromatic acids, and excreted.

The rapid conversion to the benzoic acid derivative has also been documented at high dose levels in rats. [¹⁴C]-Benzyl acetate administered by gavage to groups of male F344 rats at doses of 5, 250, or 500 mg/kg bw as the substance alone, in corn oil, or propylene glycol, resulted in excretion of 70-89% of the dose in the urine within 24 hours [Chidgey and Caldwell, 1986]. Only about 4% of the radioactivity was detected in the feces after

72 hours. Independent of the vehicle, the elimination of benzyl acetate and metabolites, was essentially complete after 3 days. No benzyl acetate was detected in the plasma or urine; however, minute amounts of benzyl alcohol were detected in the plasma. At the highest dose, benzoic acid was by far the major plasma metabolite, while at the lowest dose, conjugated benzoic acid (hippuric acid) was the major urinary metabolite. Of the metabolites, the proportion of the benzoic acid glucuronic acid conjugate increased with increasing dose, while low levels (1.0-3.6%) of free benzoic acid and benzylmercapturic acid were not affected by dose or vehicle.

To determine the effects of age on disposition of benzyl acetate, 3 to 4-, 9-, and 25-month-old F344 rats and 2-, 13-, and 25-month-old C57BI/6N mice were given a single oral dose of [¹⁴C]-benzyl acetate at doses of 5 or 500 mg/kg bw (rats) or 10 mg/kg bw (mice) [McMahon *et al.*, 1989]. In rats, approximately 80% of radioactivity was recovered in the urine in the first 24 hours for all age groups. The major urinary metabolite was hippuric acid (percentage excreted was not affected by age) and a minor urinary metabolite was benzylmercapturic acid (percentage excreted was slightly increased in 25-month-old rats). The percentage of radioactivity excreted in the feces was slightly decreased in the 25-month-old group. In mice, hippuric acid was the major urinary metabolite (93-96% of the total dose, with lower percentages excreted in 25-month-old mice than in the younger groups). Fecal excretion was a minor route of elimination and was independent of age. The authors concluded that formation of hippuric acid is not affected by age, but aging does affect the minor routes of metabolism and excretion of benzyl acetate in rats and mice.

F344 rats and B6C3F1 mice were used to study the effect of gavage versus dietary administration on the toxicokinetics of benzyl acetate [Yuan *et al.*, 1995]. Groups of F344 rats were given a single dose of 500 mg/kg bw of benzyl acetate by gavage in corn oil or were fed diets containing 2,700 ppm (approximately 648 mg/kg bw/d) benzyl acetate for 7 days. Similarly, groups of B6C3F1 mice were given benzyl acetate, 1,000 mg/kg bw by gavage in corn oil or were fed diets containing 10,800 ppm benzyl acetate (approximately 900 mg/kg bw/d) for 7 days. Plasma levels of benzyl alcohol, benzoic acid and hippuric acid were measured at 24-hour intervals. Benzyl acetate was

undetectable in the plasma after gavage (after 5 minutes in mice and 10 minutes in rats) or dietary administration. Peak plasma levels of benzoic acid and hippuric acid were reached within 3 hours of gavage administration. Compared to the gavage mode of administration, peak plasma concentrations of benzoic acid were 40-fold less in rats and 300-fold less in mice after dietary administration. Plasma concentrations of hippuric acid were similar regardless of the mode of administration. Based on the above studies it is apparent that ester hydrolysis and functional group oxidation occurs rapidly *in vivo* and is independent of dose, species, age, and mode of administration. Other studies also support the rapid *in vivo* oxidation of the benzaldehyde functional group.

Oxidation of the benzaldehyde moiety by aldehyde dehydrogenase (ALD) has been reported to yield the glycine conjugate of benzoic acid [Bray *et al.*, 1951; NTP, 1990]. In the rabbit, approximately 83 % of single doses of 350 or 750 mg/kg bw of benzaldehyde is excreted in the urine of both dose groups.

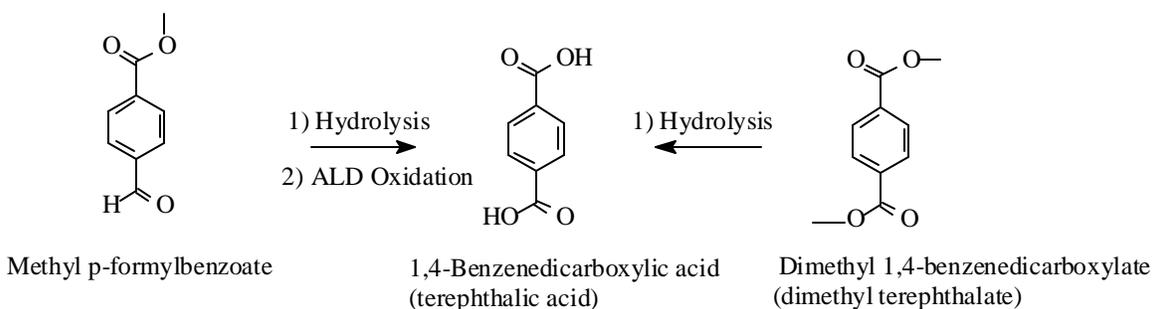
In male albino rats, approximately 100% of a 100 mg/kg bw dose of 4-hydroxy-3-methoxybenzaldehyde in a propylene glycol/water solution was excreted as free or conjugated benzoic acid derivatives in the urine within 24 hours [Strand and Scheline, 1975]. Sprague-Dawley albino rats were given 100 mg/kg bw of 4-hydroxy-3-methoxybenzaldehyde in 0.9% NaCl by intraperitoneal injection. Sixty percent of the dose was recovered in the 24-hour urine mainly as unconjugated 4-hydroxy-3-methoxybenzoic acid and the sulfate and glucuronic acid conjugates. Minor amounts of the conjugates of 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzyl alcohol, and catechol were also detected [Wong and Sourkes, 1966]. Free and conjugated forms of 4-hydroxy-3-methoxybenzoic acid identified in the urine represented greater than 80% of the dose.

Female albino rats injected intraperitoneally with 52.4 mg of 2,4-dihydroxybenzaldehyde excreted approximately 6% of the dose in the urine as the corresponding hippurate within 24 hours [Teuchy *et al.*, 1971].

Based on data for ester hydrolysis and aldehyde oxidation, methyl 4-formylbenzoate is expected to be rapidly hydrolyzed to yield 4-formylbenzoic acid that is then rapidly

oxidized (see Figure 1) to yield 1,4-benzenedicarboxylic acid (terephthalic acid). Based on pharmacokinetic evidence discussed above terephthalic acid is the predominant *in vivo* metabolite.

Figure 1



2.4.3 Absorption, Distribution, Metabolism, and Excretion

Following hydrolysis of methyl 4-formylbenzoate in the gastrointestinal tract, the corresponding benzoic acid derivative, 4-formylbenzoic acid is rapidly absorbed and oxidized primarily in the liver to terephthalic acid. Terephthalic acid is then excreted in the urine either unchanged or conjugated. Data on structurally related benzoic acid derivatives support this conclusion [Jones *et al.*, 1956; Davison, 1971; Abdo *et al.*, 1985; Temellini *et al.*, 1993].

Absorption, distribution, metabolism, and excretion studies have been conducted with various benzyl derivatives. The most relevant of these is the *in vivo* metabolite of methyl 4-formylbenzoate, terephthalic acid, and dimethyl terephthalate, a precursor of terephthalic acid. Other benzyl derivatives containing benzaldehyde and benzoate ester provide additional data on the pharmacokinetic potential and metabolic fate of methyl 4-formylbenzoate (*m*-methoxy-*p*-hydroxybenzaldehyde, benzoic acid, benzyl alcohol, 4-hydroxybenzaldehyde, 4-hydroxybenzoic acid, 2-hydroxybenzoic acid, 2,4-dihydroxybenzaldehyde, butyl *p*-hydroxybenzoate, 3,4-dimethoxybenzaldehyde,

sodium benzoate, and 2-hydroxybenzaldehyde). These substances exhibit remarkably similar patterns of pharmacokinetics and metabolism.

2.4.3.1 Rats and Mice

In female Wistar rats given a single gavage dose of 85 mg/kg bw of terephthalic acid, expired air and feces collected over 24 hours accounted for less than 0.04 and 3.3% of the total radioactivity administered, respectively [Hoshi and Kuretani, 1967]. Most of the dose was excreted in the urine within 24 hours and no other urinary metabolites were detected. The authors determined that approximately 70 and 26% of the dose was absorbed through the upper and lower gastrointestinal tract, respectively. In a separate study, female rats undergoing the same treatment were killed and blood and tissue samples were assayed for radioactivity [Hoshi and Kuretani, 1968]. After 6 hours, radioactivity was detected in plasma, kidney, liver, brain, skin, lung, pancreas, spleen, fat, heart, muscle, bone, erythrocytes, uterus, ovary, and endocrine glands. The highest concentrations were detected as follows: kidney greater than liver greater than plasma. After 48 hours, no radioactivity in these tissues was detected. A half-life of 1.2-3.3 hours was determined for terephthalic acid with first-order kinetics elimination. Oral exposure to terephthalic acid results in wide bodily distribution, but no accumulation.

The excretion of terephthalic acid following various routes of administration (gavage, intraperitoneal injection, and feeding) were studied in male rats [Hoshi and Kuretani, 1965]. Twenty-four hours following a gavage dose of 200 mg/kg bw of terephthalic acid, unchanged terephthalic acid was detected in the urine (55% of the dose) and feces (30% of the dose). After an intraperitoneal injection of 200 mg/kg bw of terephthalic acid, most of the dose was recovered in the urine within 24 hours. Feeding 300 mg/kg bw of terephthalic acid to rats resulted in excretion of 78-85% of the dose in urine and the remainder in the feces after 24 hours.

Over a period of 25 days, rats were exposed by inhalation to a particulate aerosol of 10 mg/cubic meters terephthalic acid for 6 hours/day [Amoco Corporation, 1989a]. Blood concentrations of terephthalic acid were detectable only after 10 days of exposure with progressively increasing blood levels to exposure termination reaching a high of 2.7

micrograms/ml after 25 days. After a 7-day recovery period, blood levels were less than 1 micrograms/ml.

Studies on and ester precursor of terephthalic acid, dimethyl terephthalate, indicate that the ester is hydrolyzed and the resulting acid is rapidly excreted as terephthalic acid.

Groups of rats and mice were given a single oral dose of C¹⁴-dimethyl terephthalate (amount not specified) and urine and feces were collected over a 48-hour period [Heck, 1980]. Using reverse-phase HPLC, terephthalic acid was the only urinary metabolite identified in rats; whereas, in mice, the urinary metabolites identified consisted of monomethyl terephthalate (70%), terephthalic acid (30%) and traces of unchanged dimethyl terephthalate. Similar metabolites were identified in the feces. Dimethyl terephthalate did not lower the concentration of nonprotein sulfhydryl groups, leading the authors to conclude that dimethyl terephthalate is not activated to form electrophilic metabolites.

In another oral study, rats were administered either a single dose or 5 oral doses (1 dose every other day over 10 days) of 0, 20, or 40 mg labeled dimethyl terephthalate [Moffitt *et al.*, 1975]. More than 83% of the single dose of labeled dimethyl terephthalate was excreted within 48 hours, with 86% and less than 10% of the radioactivity identified in the urine and feces at 48 hours, respectively. Following repeat dosing, greater than 91% of the total dose was identified in urine and feces within 24 hours after the final dose.

Groups of rats and mice were given a single oral dose of C¹⁴-dimethyl terephthalate (amount not specified) and urine and feces were collected over a 48-hour period [Heck, 1980]. Ninety and 10% of the dose was excreted in the urine and feces, respectively, in both species. Less than 1% remained in the animal carcasses after 48 hours.

Other benzaldehyde derivatives have also been shown to undergo rapid oxidation and excretion as the benzoic acid derivative. Male albino rats were administered the benzaldehyde derivative 4-hydroxy-3-methoxybenzaldehyde, 100 mg/kg bw in a solution of propylene glycol and water by stomach tube [Strand and Scheline, 1975]. Only trace amounts of benzoic acid derivatives remained in the urine after the first 24 hours and

none after 48 hours. Free and conjugated forms of 4-hydroxy-3-methoxybenzoic acid were the predominant metabolite excreted in the urine within 24 hours.

Sixty percent (60%) of a 100 mg/kg bw dose of 4-hydroxy-3-methoxybenzaldehyde given to Sprague-Dawley albino rats by intraperitoneal injection was recovered in the 24-hour urine mainly as unconjugated 4-hydroxy-3-methoxybenzoic acid and the sulfate and glucuronic acid conjugates of the acid conjugates [Wong and Sourkes, 1966].

Following administration of 375 mg/kg bw orally to rats or by intraperitoneal injection to mice of [¹⁴C]-benzoic acid, 88-89% of the radioactivity was recovered in the urine within 24 hours and 91-94% after 72 hours [Nutley, 1990]. Only 1-6% was present in the feces.

Female albino rats injected intraperitoneally with 52.4 mg 2,4-dihydroxybenzaldehyde excreted approximately 6% of the dose in the urine as the corresponding hippurate within 24 hours [Teuchy *et al.*, 1971].

2.4.3.2 Rabbits

Rabbits fed 1,000 mg /kg bw of 4-hydroxy-3-methoxybenzaldehyde by gavage, excreted in the urine, 69% of the dose as free and conjugated 4-hydroxy-3-methoxybenzoic acid [Sammons and Williams, 1941].

Rabbits were administered 200 mg of 3,4-dimethoxybenzaldehyde by stomach tube [Sammons and Williams, 1941]. Within 24 hours, approximately 70% of the dose was recovered in the urine as free corresponding acid (approximately 28%) and its glucuronic acid (approximately 38%) or sulfate (3-7%) conjugate.

In the rabbit, single doses of 350 or 750 mg/kg bw of benzaldehyde were excreted in the urine (approximately 83 %) by oxidation to benzoic acid and then excretion predominantly as hippuric acid (approximately 68%) [Laham *et al.*, 1988]. Other urinary metabolites identified were benzoyl glucuronic acid (10%), benzoyl glucuronide (3%), and free benzoic acid (1.5%).

2.4.3.3 *Dogs*

Groups of fasted dogs were orally administered 1,000 mg/kg bw of butyl *p*-hydroxybenzoate, or intravenously injected with 50 mg/kg bw of butyl *p*-hydroxybenzoate [Jones *et al.*, 1956]. Blood and urine samples were collected at fixed intervals until the levels returned to background levels within 48 hours. Most of the dose was recovered between 6 and 30 hours after dosing as the *p*-hydroxybenzoic acid conjugate of glucuronic acid at 48% and 40% for the oral and intravenous route, respectively. Although the relatively low rate of recovery seen in both dosing methods was attributed to incomplete hydrolysis of the ester in the body, *in vitro* incubation of the butyl ester with freshly prepared liver homogenate showed complete hydrolysis within 30-60 minutes. Studies conducted with other related benzoate esters, such as methyl and ethyl *p*-hydroxybenzoate, showed significantly higher rates of material recovery suggesting that an increase in the homologous series of alkyl esters may result in the activation of other metabolic and excretion pathways. Overall, the authors concluded that butyl *p*-hydroxybenzoate and other alkyl esters are readily absorbed, metabolized, and excreted by the body.

2.4.3.4 *Humans*

In humans, 4 full-term and 9 pre-term infants were administered intravenous or intramuscular doses of 0.007-0.222 mmol/kg bw of benzyl alcohol in medication [LeBel *et al.*, 1988]. Pre-term infants had maximum serum concentration levels of benzoic acid approximately 10 times those in full-term infants. Benzoic acid was found at higher percentages in the plasma than hippuric acid regardless of administration route in pre-term infants compared to term infants indicating that glycine conjugation is deficient in pre-term compared to full-term infants.

In humans receiving oral doses of 40, 80, and 160 mg/kg bw of sodium benzoate, the clearance of benzoic acid increased disproportionately to dose while the clearance for hippuric acid was proportional to dose [Kubota *et al.*, 1988; Kubota and Ishizaki, 1991]. Peak plasma concentrations of benzoic acid increased with increasing dose, while peak

hippuric acid concentrations did not change. The data suggest that the conjugation with glycine to form hippuric acid is a saturable process in humans.

Doses of 2,000-5,000 mg sodium benzoate were orally administered to male volunteers [Amsel and Levy, 1969]. At 5,000 mg, a 5,000 mg glycine supplement was administered one hour later and 2,000 mg supplements were given every 2 hours thereafter. Benzoate was excreted mainly as hippuric acid. No free benzoic acid was detected. Minor amounts of benzoyl glucuronide were detected, with more formed at the highest dose. Glycine supplementation increased the rate of hippuric acid excretion, indicating that at high dose levels, glycine is rate limiting for formation of hippuric acid.

Administration of daily oral doses of 5,330-6,000 mg 2,4-dihydroxybenzoic acid in 1,000 mg doses every 3 hours for 2 to 16 days to patients for the treatment of rheumatic fever resulted in average daily urinary excretion rates of 42.7-75.8 % [Clarke *et al.*, 1958]. Average daily excretion of sulfate conjugate was relatively constant during the study, but average daily excretion of glucuronic acid conjugate increased 4- to 6-fold over the 16-day period.

In humans, an oral dose of 100 mg 4-hydroxy-3-methoxybenzaldehyde dissolved in water revealed an increase in the 4-hydroxy-3-methoxybenzoic acid output in the urine from a background level of 0.3 mg/24 hours to 96 mg/24 hours [Dirscherl and Wirtzfeld, 1964]. The observed increase was approximately 94% of the parent aldehyde dose.

2.4.3.5 Fish

Pharmacokinetic and metabolic studies have been performed on two fish species. In channel catfish, intravascular (iv) or peroral administration of a 10 mg/kg dose of [¹⁴C]-benzoic acid was rapidly absorbed and eliminated. After iv dosing, elimination half-life was 5.9 hours, total body clearance was 61 ml/min., and volume of distribution was 369 ml/hr/kg. After oral administration, absorption half-life was only 0.8 hours and bioavailability was greater than 95%. Greater than 80% of the iv dose was excreted *via* the renal pathway within 24 hours. The major excreted metabolite was the taurine conjugate of benzoic acid [Plakis and James, 1990]. Being a more polar acid, terephthalic

acid, the *in vivo* metabolite of methyl 4-formylbenzoate is anticipated to undergo even more rapid excretion.

In the southern flounder, greater than 95% of a 15 mg dose of [¹⁴C]-benzoic acid given by intramuscular injection was excreted as the taurine conjugate of benzoic acid in the urine. [James and Pritchard, 1987]. The rate of excretion was slow, approximately 10% per day. A subsequent investigation of the transport of benzoic acid, benzoyltaurine, and hippuric acid revealed that, at 100 uM, conjugation of benzoic acid with taurine was slow and there was also saturation of the transport of benzoyltaurine by isolated renal tubules. The amino acid conjugation (e.g., taurine) of benzoic acid has also been studied in rainbow trout (*Salmo gairdneri*) [Burke *et al.*, 1987]. Greater than 99% of the radioactivity derived from a 10 mg/kg dose of [¹⁴C]-benzoic acid was given by gelatin capsule was excreted in the urine within 48 hours. Greater than 98% of the excreted radioactivity was accounted for by a single metabolite, benzoyltaurine. Based on these studies, it is concluded that once benzoic acid has been absorbed by fish, is rapidly excreted as the taurine conjugate.

2.4.3.6 Multiple Species

Hippuric acid was the primary urinary metabolite following oral administration of 1-400 mg/kg bw of [¹⁴C]-benzoic acid to various species including primates, pigs, rabbits, rodents, cats, dogs, hedgehogs, bats, birds, and reptiles [Bridges *et al.*, 1970]. The ornithine conjugate of benzoic acid, ornithic acid, was the major urinary metabolite excreted within 24 hours in chickens and reptiles. Benzoyl glucuronide was predominant in the fruit bat. In humans, greater than 99% of ¹⁴C was excreted as hippuric acid within 24 hours.

Based on extensive data for terephthalic acid and its precursor, various benzoate and benzaldehyde derivatives, it is concluded that methyl 4-formylbenzoate is hydrolyzed to 4-formylbenzoic acid that is then oxidized rapidly *in vivo* to yield terephthalic acid. This acid is the predominant plasma metabolite, which is rapidly excreted either free or conjugated in the urine. Therefore, data on the principal metabolite (terephthalic acid) and a precursor (dimethyl terephthalate) of this metabolite is directly related to the hazard

assessment of methyl 4-formylbenzoate. Also, toxicologic data on the mono-functional analogs (methyl benzoate and benzaldehyde) that participate in the same metabolic pathways of detoxication present a conservative estimate of toxic potential for methyl 4-formylbenzoate. These data have also been included in the test plan and robust summaries.

3 TEST PLAN

3.1 PHYSICAL AND CHEMICAL PROPERTIES

3.1.1 Melting Point

Literature values from reliable sources are available for methyl 4-formylbenzoate and its related substances. For methyl 4-formylbenzoate, the reported value of 60-62 °C [Aldrich Chemical Co., 1986] and the calculated value of 40.11 °C [MPBPWIN EPI Suite, 2000] are in reasonable agreement. Measured melting points for the principal *in vivo* metabolite terephthalic acid is greater than 300 to 425 °C [Bemis *et al.*, 1982; Anon, 1988; ICI Chemicals & Polymers, Ltd., 1991; CRC Handbook, 2000].

3.1.2 Boiling Point

For methyl 4-formylbenzoate, the reported value of 265 °C [Aldrich Chemical Co., 1986] and the calculated value of 261.31 °C [MPBPWIN EPI Suite, 2000] confirm consistency.

3.1.3 Vapor Pressure

Based on the input parameters of reported boiling point (265 °C) and melting point (61 °C), the calculated vapor pressure for methyl 4-formylbenzoate is 0.00662 mm Hg (0.00088 kPa) at 25 °C [MPBPVWIN EPI Suite, 2000]. The measured value for a structurally related substance dimethyl terephthalate, in which the aldehyde group of methyl 4-formylbenzoate is replaced by a carbomethoxy group, is reported to be in a similar range (0.01 mm Hg at 25 °C or 0.00133 kPa) [Eastman Chemical Co., MSDS]. The vapor pressure of methyl 4-formylbenzoate is expected to be significantly lower than the reported vapor pressure for the mono-functional aldehyde benzaldehyde (1.27 mm Hg or 0.169 kPa) [Ambrose *et al.*, 1975] and the monofunctional ester methyl benzoate (0.38 mm Hg or 0.0507 kPa) [Daubert and Danner, 1986]. Therefore, the measured vapor pressure of methyl 4-formylbenzoate is expected to be in the range of 0.001 and 0.01 mm Hg.

3.1.4 Octanol/Water Partition Coefficients

The calculated log Kow value for methyl 4-formylbenzoate is 1.55 [KOWWIN EPI Suite, 2000]. A measured Kow for the less polar structurally related substance dimethyl terephthalate is reported to be of 2.25 [Hansch *et al.*, 1995] while the measured Kow values for the more polar substance terephthalic acid is in the range between 1.16 and 2.0 [Church, undated; Leo, 1978; Tomida *et al.*, 1978; Dunn and Johnson, 1983; Chan and Hansch, 1985]. Given these data the anticipated log Kow value for methyl 4-formylbenzoate is in the range from 1.16 to 1.55.

3.1.5 Water Solubility

The calculated water solubility value for methyl 4-formylbenzoate is 3,136 mg/L [WSKOWWIN EPI Suite, 2000]. Whereas, the measured values for terephthalic acid are in the range from 15 to 19 mg/L at 10-25 ° C [ICI Chemicals & Polymers, Ltd., 1991; Bemis *et al.*, 1982]. A higher but somewhat wider range of water solubility is reported for dimethyl terephthalate. Reported water solubilities exhibit a range of values from 19 to 37.2 mg/L [Montefibre Spa, undated; Kuhne, R. *et al.*, 1995; Eastman Kodak Co. Environmental Safety Data Sheet; undated].

3.1.6 New Testing Required

No additional testing recommended

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

The calculated half-life for reaction of methyl 4-formylbenzoate with hydroxyl radicals is 7.371 hours [AOPWIN EPI Suite, 2000]. Given that the aldehyde function is more photochemically reactive than a methyl ester function, the half-life of methyl 4-formylbenzoate is anticipated to be less than that of dimethyl terephthalate. This is the case. The measured half-life of dimethyl terephthalate is approximately 3 days [Brown *et al.*, 1975]. In addition, the photo-oxidation half-life of dimethyl terephthalate was reported to be 4.7 to 46.6 days [Howard *et al.*, undated]. It is concluded that the half-life of methyl 4-formylbenzoate is less than 1 day.

3.2.2 Stability in Water

The hydrolysis of methyl 4-formylbenzoate has been measured over the pH range of 4 to 9. Greater than 50% hydrolysis to yield 4-formylbenzoic acid was observed after 2.4 hours at pH=9, indicating the half-life of the ester is less than 1 day at slightly basic pH. The estimated half-life at pH=7 is estimated to be approximately 200 days [Hoffman, 2003]. These experimental data are in good agreement with calculated hydrolysis data. Hydrolysis of methyl 4-formylbenzoate to yield 4-formylbenzoic acid has a half-life of 23.9 days at pH=8 and 239 days at pH=7 [HYDROWIN EPI Suite, 2000]. The measured half-life of dimethyl terephthalate in water has been reported as 1 to 4 weeks in surface water, 2 to 8 weeks in ground water and 321 days in neutral water [Howard *et al.*, undated; Mabey and Mill, 1978].

3.2.3 Biodegradation

Methyl 4-formylbenzoate was predicted to be readily degradable by model calculations [BIOWIN EPI Suite, 2000]. Measured values of biodegradability data for terephthalic acid, benzaldehyde, and methyl benzoate indicate all substances are readily and ultimately biodegradable using a standard OECD 301B test or 301F protocol [Gerike and Fischer, 1979; Quest International Ltd., 1995; Corby, 1995; Amoco Chemicals Co., 1992;

Amoco Corporation, 1991]. The parent acid benzoic acid and its sodium salt were readily biodegradable in a COD (chemical oxygen demand) test [Birch and Fletcher, 1991; Pitter, 1976]. Since hydrolysis of the esters and ready oxidation of the aldehydes yields corresponding acid derivatives, the data on benzoic acid and its sodium salt validate the observations that terephthalic acid (the product of aldehyde oxidation and ester hydrolysis) is also readily biodegradable.

3.2.4 Fugacity

Transport and distribution in the environment were modeled using a combination of ECOSAR Level III EPI Suite [ECOSAR EPI Suite, 2000] and Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11 [Trent University, 1999]. The principal measured input parameters into the Trent University model are molecular weight and melting point. When measured values were unavailable, calculated data were used [ECOSAR EPI Suite, 2000]. Calculated input parameters included vapor pressure [MPBPVPWIN EPI Suite, 2000] and water solubility [WSKOWWIN EPI Suite, 2000].

The model predicts that methyl 4-formylbenzoate (1000 kg, assumed daily emission rate) is distributed mainly to the water (greater than 95%). Consistent with the high water solubility and log Kow data the distribution to the soil was only 3%. Less than 1% was distributed to air and sediment. Based on this physiochemical model, the ratio for distribution of the methyl 4-formylbenzoate between water (greater than 95%) and fish (0.00017 %) is greater than four orders of magnitude suggesting low bioaccumulation in fish.

The significance of these calculations must be evaluated in the context that methyl 4-formylbenzoate is readily hydrolyzed and oxidized to yield terephthalic acid. The model does not account for the influence of this chemical reactivity on partitioning in the environment. Therefore, the relevance of fugacity calculations for methyl 4-formylbenzoate must be evaluated in the context of these factors.

3.2.5 New Testing Required

None required.

3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

Based on aldehyde ECOSAR Class, 96-hour LC50 for methyl 4-formylbenzoate was calculated to be 19 mg/L. Based on ester ECOSAR Class, 96 hour LC50 was calculated to be 43 mg/L. Experimental LC50 data available on the structural relatives are in the same range as the calculated LC50 for methyl 4-formylbenzoate.

Dimethyl terephthalate exhibited 96-hour LC50 values in the same range as the values calculated for methyl 4-formylbenzoate. In static tests with fathead minnows, the 96-hour LC50 for dimethyl terephthalate was reported to be in the range from 9.6 to 45 mg/L [Eastman Kodak Co., 1977; 1984].

Data on the mono-functional analogs, methyl benzoate and benzaldehyde provide a basis for evaluating the toxicity of each functional group. Methyl benzoate also exhibits similar aquatic toxicity to fish. The 96-hour LC50 in Bluegill sunfish was 28.3 mg/L with a no observable effect concentration (NOEC) or 10 mg/L based on mortality and loss of equilibrium [Cunningham, 1997a]. Also, the 96-hour LC50 value for methyl benzoate is approximately 18 mg/L [ECOSAR EPI Suite, 2000]. For benzaldehyde, the calculated 96-hour LC50 model value was 13 mg/L [ECOSAR EPI Suite, 2000]. The NOEC for benzaldehyde of 1-day and 4-day larvae was reported to be less than 0.9 mg/L and the NOEC for survival of 1-day and 4-day larvae was 3.6 mg/L (first test) while the NOEC for survival in second test was 0.22 mg/L for 1-day larvae and 1.8 mg/L for 4-day and 7-day larvae [Pickering *et al.*, 1996]. In a poorly documented study, a 14-day LC50 for benzaldehyde in guppies has been reported to be 1.57 micromoles/L (0.17 mg/L) [Deneer *et al.*, 1988].

Since the experimental data on structural relatives and the calculated values of methyl 4-formylbenzoate are in good agreement, it is anticipated that the model 96-hour LC50

value of approximately 19 mg/L calculated for methyl 4-formylbenzoate is reliable [ECOSAR EPI Suite, 2000]. It is anticipated that the toxicity of methyl 4-formylbenzoate is less than or similar to that of the mono-functional analogs, methyl benzoate and benzaldehyde.

3.3.2 Acute Toxicity to Aquatic Invertebrates

Acute toxicity for aquatic invertebrates parallels that for fish exhibiting low experimental for toxicity. The calculated 48-hour LC50 for methyl 4-formylbenzoate in *Daphnia magna* is 16.4 mg/L [ECOSAR EPI Suite, 2000]. Experimental data for dimethyl terephthalate (methyl 1,4-dibenzoate) exhibits a similar order of toxicity. In *Daphnia magna*, the 48-hour static EC50 and LC50 were reported to be greater than 30 and 30.4 mg/L, respectively [Eastman Kodak Co., 1984; DuPont Chemicals, MSDS undated]. In *Daphnia magna*, the 96-hour static LC50 values were reported to be up to greater than 100 mg/L [Eastman Kodak Co., 1977]

Data for mono-functional analogs support a low order of toxicity for methyl 4-formylbenzoate. The 48-hour EC50 for methyl benzoate has been reported to be 32.1 mg/L in *Daphnia magna* [Cunningham, 1997b]. The 48-hour LC50 model value for methyl benzoate was calculated to be 84 mg/L [ECOSAR EPI Suite, 2000]. The 24-hour LC50 for benzaldehyde to *Daphnia magna* has been reported as 50 mg/L [Bringmann and Kuehn, 1977]. The calculated 48-hour LC50 model value for benzaldehyde was 12 mg/L [ECOSAR EPI Suite, 2000].

Experimental data adhering to OECD Guideline 202 are available for terephthalic acid a reaction product of methyl 4-formylbenzaldehyde. In a static test with *Daphnia magna*, the 48-hour EC50 and the NOEC for terephthalic acid were reported to be greater than 1,000 (nominal) mg/L and 600 (nominal) mg/L, respectively [Amoco Corporation, 1993b].

Based on the calculated EC50 value for methyl 4-formylbenzoate and the experimental EC50 and LC50 for methyl benzoate, benzaldehyde, and dimethyl terephthalate, it is concluded that methyl 4-formylbenzoate exhibits an EC50 in the range of 10-50 mg/L.

As for fish toxicity, it is also anticipated that the toxicity of methyl 4-formylbenzoate is less than or similar to that of the mono-functional analogs, methyl benzoate and benzaldehyde.

3.3.3 Acute Toxicity to Aquatic Plants

The model 96-hour EC50 for methyl 4-formylbenzoate was calculated to be 204 mg/L [ECOSAR EPI Suite, 2000]. In GLP-quality studies, terephthalic acid and dimethyl terephthalate exhibit very low and moderate toxicity, respectively, in aquatic plants. In a static growth inhibition study with algae conducted under OECD Guideline 201, the 96-hour EC50 and NOEC for terephthalic acid were both greater than 1000 (nominal) mg/L [Amoco Corporation, 1993c]. For dimethyl terephthalate, the 72-hour EC50 for algae growth in an EEC Directive 88/302 study was reported to be greater than the highest concentration tested (32.3 mg/L) and the NOEC was 10.8 mg/L [Huls AG, 1993].

Benzaldehyde stimulated the respiration of cells from *Chlorella vulgaris* in a dose dependent manner, but inhibited growth 30% after 80 hours and 10% after 160 hours at a concentration of 17 mg/L [Dedonder and Van Sumere, 1971]. Oxygen uptake was maximal at lower pH (pH 5.6 versus 7.2) and higher concentrations (0.001 M or 170 mg/L). The 96-hour EC50 model values for benzaldehyde and methyl benzoate were 152 and 1.4 mg/L, respectively [ECOSAR EPI Suite, 2000].

Based on the low orders of toxicity for terephthalic acid, dimethyl terephthalate and the mono-functional analog benzaldehyde, it is concluded that EC50 of methyl 4-formylbenzoate in algae is in the range of 20 to 200 mg/L.

3.3.4 New Testing Required

There are sufficient fish and invertebrate acute toxicity data for the structurally related substances and mono-functional analogs that are consistent with model values. No additional studies are recommended.

Acute plant toxicity studies for terephthalic acid, dimethyl terephthalate, and benzaldehyde indicate EC50 values greater than 20 mg/L. The calculated value of 204

mg/L supports a low order of toxicity to aquatic plants. No additional studies are required for methyl 4-formylbenzoate.

3.4 HUMAN HEALTH DATA

Hazard assessment data is presented on methyl 4-formylbenzoate, its principal *in vivo* metabolite (1,4-benzenedicarboxylic acid or terephthalic acid), a structurally related substance (dimethyl terephthalate) that also forms 1,4-benzenedicarboxylic acid *in vivo*, and two substances that are mono-functional analogs of methyl 4-formylbenzoate, benzaldehyde and methyl benzoate. Based on extensive database of information (WHO Technical Information Series, 2001) it is anticipated that the toxicity of methyl 4-formylbenzoate is less than or similar to that of the mono-functional analogs.

3.4.1 Acute Toxicity

Oral and intraperitoneal LD50 values are available for methyl 4-formylbenzoate. In rats and mice, the oral LD50 values ranged from 1600 to 3200 mg/kg bw demonstrating the low toxicity of this substances. Similar oral LD50 values have also been reported in rats and guinea pigs for benzaldehyde and methyl benzoate and in mice and rabbits for methyl benzoate [Graham and Kuizenga, 1945; Smyth *et al.*, 1954; Sporn *et al.*, 1967; Jenner *et al.*, 1964; Kravets-Bekker and Ivanova, 1970]. High oral LD50 values (greater than 5000 mg/kg bw) have been reported in rats orally exposed to terephthalic acid and dimethyl terephthalate [Krasavage *et al.*, 1973; Amoco Corporation, 1990].

Oral LD50 studies are available for methyl 4-formylbenzoate and its structural relatives. Most of the data were obtained prior to GLP and OECD Guidelines. However, the data mutually confirm that methyl 4-formylbenzoate exhibits a low order of acute toxicity. No further studies of acute toxicity are recommended.

3.4.2 Genetic Toxicity

Overall, *in vitro* and *in vivo* genotoxicity studies have been conducted with a variety of aromatic substances substituted with methyl ester and aldehyde functional groups. Data exist for the mono-functional analogs, benzaldehyde and methyl benzoate as well as for the difunctional analogs, terephthalic acid and methyl terephthalate. The results of these studies were predominantly negative, as described below. Most importantly, *in vivo*

studies have all yielded negative results. These negative *in vivo* genotoxicity assays are supported by the lack of tumorigenicity in chronic animal studies with members of this group. Therefore, the database on genetic toxicity of the structurally related benzyl derivatives is adequate to support the low genotoxic potential of methyl 4-formylbenzoate.

3.4.2.1 In Vitro

In vitro genetic toxicity data are available on structurally related substances, benzaldehyde, methyl benzoate, terephthalic acid, and dimethyl terephthalate. The vast majority of standardized *in vitro* genotoxicity assays (Ames (AMS), mouse lymphoma (MLA), sister chromatid exchange (SCE), chromosomal aberration (ABS), and unscheduled DNA synthesis (UDS)) show no evidence of genotoxicity.

The above substances were non-mutagenic in all standard plate incorporation and/or pre-incubation Ames assays using *Salmonella typhimurium* strains TA92, TA94, TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538, when tested at concentrations ranging up to the level of cytotoxicity or at ICH/OECD-recommended maximum test concentrations, both in the absence and presence of metabolic activation (S9 fraction) [Sasaki and Endo, 1978; Florin *et al.*, 1980; Rapson *et al.*, 1980; Kasamaki *et al.*, 1982; Zeiger *et al.*, 1982, 1992; Wiessler *et al.*, 1983; Heck *et al.*, 1989; NTP, 1990; Monarca *et al.*, 1989; 1991; Dillon *et al.*, 1992, 1998]. Additional Ames tests on metabolites isolated from the urine of rats administered benzaldehyde by oral gavage also were negative in *Salmonella typhimurium* strains TA98 and TA100, both with and without metabolic activation [Rockwell and Raw, 1979]. Mutation or DNA repair assays using *Escherichia coli* strains WP2 uvrA or Sd-4-73 with methyl benzoate [Szybalski, 1958] also showed no evidence of genotoxicity.

Negative results were obtained with benzaldehyde in the Rec DNA repair assay using *Bacillus subtilis* strains H17 and M45, whereas weakly positive results were obtained with metabolically activated benzaldehyde [Oda *et al.*, 1978; Matsui *et al.*, 1989].

In vitro assays in isolated mammalian cells produced both negative and positive results. Benzaldehyde exhibited evidence of mutagenicity in the forward mutation assay with L5178Y mouse lymphoma cells (MLA), both with and without metabolic activation [McGregor *et al.*, 1991; Heck *et al.*, 1989]. Dimethyl terephthalate did not alter mutation frequency with or without metabolic activation [Myhr and Caspary, 1991].

In cytogenetic tests performed in Chinese hamster ovary and fibroblast cell lines, both positive and negative results were reported with benzaldehyde, with the positive result usually occurring at the higher culture concentrations in the ABS assay [Kasamaki *et al.*, 1982; Sofuni *et al.*, 1985; Galloway *et al.*, 1987]. Both dimethyl terephthalate and terephthalic acid produced negative results when tested in the ABS assay using human peripheral blood lymphocytes [Monarca *et al.*, 1989; 1991]. Dimethyl terephthalate was also negative in CHO cells in the presence or absence of metabolic activation [Loveday *et al.*, 1990].

The authors of the MLA and ABS assays [Heck *et al.*, 1989] have emphasized that the positive results in the MLA and ABS assays may be artifacts resulting from changes in culture pH and osmolality. Treatment with high dose levels of substances (e.g., reactive aldehydes and carboxylic acids) with the potential to alter acidity or osmolality may induce a significant increase in mutant frequencies or aberrations in these assays. Often the results are inconsistent with the results of other genotoxicity assays (i.e., AMS and UDS) [Heck *et al.*, 1989].

In the SCE assay, equivocal results were reported for benzaldehyde in CHO cell lines and in human lymphocytes [Galloway *et al.*, 1987; Sasaki *et al.*, 1989; Jansson *et al.*, 1988]. Negative results were obtained in this assay for dimethyl terephthalate (CHO cells) [Jansson *et al.*, 1988; Loveday *et al.*, 1990].

No UDS was observed in rat hepatocytes exposed to benzaldehyde [Heck *et al.*, 1989] nor in human HELA or hamster embryo cells exposed to dimethyl terephthalate [Monarca *et al.*, 1989; 1991].

In summary, data available for approximately 30 *in vitro* genotoxicity assays for structural relatives or metabolites of methyl 4-formylbenzoate indicate a low genotoxic potential for this chemical.

3.4.2.2 *In Vivo*

In vivo mutagenicity and genotoxicity data exist for the structurally related substances (terephthalic acid, dimethyl terephthalate, and benzaldehyde).

None of these substances showed any evidence of genotoxicity in well-recognized *in vivo* assays (mouse micronucleus and sex-linked recessive lethal). In mammals, substances were administered by intraperitoneal injection at doses that were significant fractions of the reported lethal dose levels.

Mouse micronucleus tests were consistently negative with dimethyl terephthalate and terephthalic acid [Shelby *et al.*, 1993; Bioreliance, 2001] with the exception of one study testing dimethyl terephthalate administered in DMSO at high dose levels [Goncharova *et al.*, 1988]. The positive finding in the Goncharova *et al.* (1988) study was considered by the authors to be unusual and perhaps related to a reaction between the solvent vehicle (DMSO) and the test material (dimethyl terephthalate).

In the (sub-mammalian) sex-linked recessive lethal mutation assay in fruit flies (*Drosophila melanogaster*), negative results were obtained with dimethyl terephthalate and benzaldehyde either after feeding or administration by intraperitoneal injection [Woodruff *et al.*, 1985].

Given that the *in vitro* and *in vivo* results both consistently demonstrate that the substances related to methyl 4-formylbenzoate exhibit a low order of genotoxic potential, no additional studies are required for this chemical.

3.4.3 Repeated Dose Toxicity

Studies examining possible subacute and subchronic toxic effects are available on the principal metabolite (terephthalic acid), dimethyl terephthalate, benzaldehyde, and methyl benzoate. Taken together, these data indicate a low order of subchronic toxicity for methyl 4-formylbenzoate. Long term studies on dimethyl terephthalate, benzaldehyde, benzoic acid and its sodium salt provide adequate evidence that methyl 4-formylbenzoate, its principal metabolite, related substances (dimethyl terephthalate), and mono-functional analogs all exhibit no significant carcinogenic potential in animals.

3.4.3.1 *Subacute Studies*

3.4.3.1.1 *Rats*

In preliminary dose range-finding studies [NTP, 1990], benzaldehyde was administered to rats by corn oil gavage for 14-16 days at doses up to 2,000 mg/kg bw/day. The no-observed-adverse-effect level (NOAEL), determined from this study based on increased mortality, was 400 mg/kg bw/day for benzaldehyde.

In addition to oral exposure, benzaldehyde has been tested in rats through inhalation. The 14-day inhalation study [Laham *et al.*, 1991] with benzaldehyde showed overall, mild irritation of the nasal mucosa, effects of the central nervous system, and some hematological and biochemical effects at exposures between 500 and 1,000 ppm was reported. Exposed male rats, but not female rats, showed goblet cell metaplasia thought to be related to adaptation following exposure to benzaldehyde.

Dimethyl terephthalate was fed to rats for 14 days at dietary concentrations of up to 3% resulting in an increase in the incidence of bladder calculi at the higher doses [Chin *et al.*, 1981]. Rats with calculi had grossly observable irregular thickening of the bladder wall. Bladder calculi were composed of calcium and terephthalic acid with 5-7% protein. Similar effects were reported in F344 rats maintained on a diet of terephthalic acid [Chin *et al.*, 1981].

In a 28-day inhalation study, rats exposed to 21.5 mg terephthalic acid/m³ for 6 hours/day, 5 days/week showed no adverse effects [Amoco Corporation, 1973].

3.4.3.1.2 Mice

The preliminary testing conducted by the National Toxicology Program (NTP) also included a gavage mouse study with benzaldehyde [NTP, 1990]. Mice were administered benzaldehyde for 14-16 days at doses of up to 3,200 mg/kg bw/day. Based on increased mortality, the NOAEL was determined to be 400 mg/kg bw/day for benzaldehyde.

3.4.3.2 Subchronic Studies

3.4.3.2.1 Rats

When benzaldehyde was administered by gavage to rats for 13 weeks [NTP, 1990], survival and body weights were reduced at the highest dose tested (800 mg/kg bw/day). Also at the high dose, multiple histopathological effects were reported in the brain, forestomach, liver, and kidney. Based on the various lesions reported at the high dose, the NOAEL was determined to be 400 mg/kg bw/day and doses selected for a 2-year study were 200 and 400 mg/kg bw/day.

In addition to the NTP study, benzaldehyde has been tested in 2 other studies [Sporn *et al.*, 1967; Hagan *et al.*, 1967] reported with limited experimental detail and prior to the establishment of GLP guidelines. In a study [Sporn *et al.*, 1967], adult white rats were orally administered doses of 10 mg benzaldehyde diluted in 0.1 ml of oil on alternate days for a period of 8 weeks. No hepatic enzyme activity was reported. The authors conducted a second investigation lasting 12 weeks in which no effects on growth, liver or adrenal gland weight were reported. In a study conducted by the FDA, benzaldehyde was fed to groups of Osborne-Mendel rats at concentrations of 0 or 10,000 ppm (approximately 500 mg/kg bw/day) for 16 weeks or 0 or 1,000 ppm (approximately 50 mg/kg bw/day) for 27-28 weeks [Hagan *et al.*, 1967]. No clinical, hematological or histopathological effects were reported.

Methyl benzoate administered to rats (route not specified) for 45 days at doses of 111 or 500 mg/kg bw/day, did not produce any histological findings, but was reported to produce some effects on blood parameters [Kravets-Bekker and Ivanova, 1970].

Rats were fed 0, 0.05, 0.16, 0.50, 1.6, or 5.0% terephthalic acid in the diet for 15 weeks [Amoco Corporation, 1970]. There were no effects on feed intake, hematology, clinical chemistry, or organ weights. At the highest dose, 3 females died (cause unknown), body weights were mildly depressed, hematuria was noted on a sporadic basis in males, "small" occult blood was reported during urinalysis in both sexes, increased incidence of bladder calculi in males was noted, and significantly increased incidence and severity of proliferative changes (hyperplasia) in the urinary bladder and occasionally kidney pelvis epithelium of males was reported. One male rat at the mid-dose also died (cause unknown). The microscopic pathology, although noted in all groups (including controls), was only statistically significant in high-dose males. A NOAEL of 1.6% (approximately 1220 and 1456 mg/kg bw for males and females, respectively) reported by the authors was based on bladder calculi and subsequent hyperplasia.

In a 90-day dietary study, rats were initially fed 5% terephthalic acid for the first week of the study, but it was reduced to 3% for the remainder of the study [Amoco Corporation, 1972]. Bladder stones were reported in 11/18 males and 3/19 females. In addition, 13/18 males and 3/19 females were reported to have mild to moderate hyperplasia of the bladder urothelium. Eight out of 13 males (62%) and 3/3 (100%) females with transitional cell hyperplasia also had bladder stones consisting of calcium terephthalate and protein.

3.4.3.2.2 *Mice*

Mortality was also increased (9/10 males and 1/10 females died) when benzaldehyde was administered by corn oil gavage to B5C3F1 mice at the highest dose tested (1,200 mg/kg bw/day) [NTP, 1990]. At 600 mg/kg bw/day, the final mean body weight of males was 9% lower than controls. Mild to moderate renal tubule degeneration occurred in all males in the high-dose group and in 1/10 males in the 600 mg/kg bw/day group. No other

compound-related effects were reported. Based on the mild renal lesions and depressed body weight gain, the doses selected for a 2-year study were 300 and 600 mg/kg bw/day.

3.4.3.3 Chronic Studies

3.4.3.3.1 Rats

Benzaldehyde was administered at dose levels of 300 or 600 mg/kg bw in corn oil by gavage five days per week to groups of 50 F344/N rats or B6C3F1 mice for a period of 2 years [NTP, 1990]. Animals were observed once weekly for 12-13 weeks and at least monthly thereafter. All animals were subject to necropsy after death, or at the end of the study. Throughout these studies, mean body weights were comparable among all groups. Increased mortality seen in some of the groups was attributable to the gavage procedure, reflux and aspiration of the gavage material into the lungs, or administration errors resulting in direct disposition of material into the lungs. Summaries of the rat studies and results are described below and the results of the mouse studies are discussed in the section on mice.

Groups of 50 male and 50 female F344/N rats were administered 0, 200, or 400 mg/kg bw/day, 5 days/week, of benzaldehyde in corn oil by gavage for 2 years. Survival was significantly decreased in the high-dose male group after Day 373; however, there were no other compound-related effects in any of the rats. The NTP concluded that there was “no evidence of carcinogenic activity” in rats given benzaldehyde under these study conditions [NTP, 1990].

White and gray rats were administered 0.005 or 0.05 mg/kg bw/day of methyl benzoate for 6 months [Kravets-Bekker and Ivanova, 1970]. The general condition of the animals in both dose groups did not differ from controls. At the high dose, there was decrease in the number of reticulocytes (p less than 0.01) but there was no difference from controls in prothrombin time or phagocytic activity at either dose. In behavioral tests, at the high dose, the latent period for response to "bell" or "light" stimulus was increased. Also, there was an increase in the number of sulfhydryl groups in cerebral tissue of high-dose rats. At

necropsy, congestion and swelling of the hepatic central veins and capillaries was reported in high-dose rats. There were no histological findings in the low-dose animals.

In a standard cancer bioassay Fischer 344 rats were fed up to 5000 ppm dimethyl terephthalate in the diet for 2 years with no adverse effects (Federal Register Notice, 1981 – no robust summary included).

Rats fed up to 1000 mg/kg bw/day of terephthalic acid in the diet for 2 years showed no adverse effects with the exception of bladder stones in 13/126 high-dose females [Chemical Industry Institute of Technology (CIIT), 1983]. Through scheduled sacrifices at 6, 12, and 18 months, the progression to the development of the calculi was observed. No evidence of bladder stones were observed at 6 and 12 months; however, sand-like particle or bladder calculi were reported in 2 of the high-dose females at 18 months.

Similar results were reported in another 2-year rat study in which rats were fed up to 5% terephthalic acid [Gross, 1974]. In this study, the highest dose (approximately 2500 mg/kg bw/day) was reported to produce reduced body weight gain in both sexes, increased kidney weight in males, increased adrenal weight in both sexes, increased incidence of bladder stones (42/47 males; 39/42 females), and increased incidence of bladder and ureter tumors (21/37 males; 21/34 females). At the 2% concentration (approximately 1000 mg/kg bw/day), there was reduced body weight gain in males, and reduced liver, kidney, and heart weight in females. The incidence of bladder stones in females fed 1% was 1/48. Bladder and ureter tumors were reported as 1/48 at 2% in males, 2/48 at 2% in females and 1/43 at 1% in males.

3.4.3.3.2 *Mice*

Groups of 50 male B6C3F1 mice were administered 0, 200, or 400 mg/kg bw/day, 5 days/week of benzaldehyde in corn oil by gavage for 2 years. Groups of 50 female mice were administered 0, 300, or 600 mg/kg bw/day of benzaldehyde. There were no compound-related clinical signs or effects on body weight, and survival was not affected by treatment. A non-statistically significant increase in the incidence of forestomach focal hyperplasia was reported in both sexes. An increase in the incidence of squamous cell

papillomas of the forestomach was reported in both sexes at all doses tested and reached statistical significance in the female mice. There was no increase in the incidence of squamous cell carcinomas of the forestomach in either sex. The NTP considered the increase in forestomach papillomas to be attributable to a concurrent increase in hyperplasia as a result of benzaldehyde treatment and, therefore, concluded that there was “some evidence of carcinogenic activity” in mice under these study conditions [NTP, 1990].

The occurrence of squamous cell papillomas and forestomach hyperplasia in rodents is common in NTP bioassay gavage studies in which a high concentration of an irritating material in corn oil is delivered daily by needle into the forestomach for two years. High concentrations of aldehydes such as malonaldehyde, furfural, and benzaldehyde (NTP 1988, 1990, 1993 - no robust summaries prepared) and other irritating substances including dihydrocoumarin and coumarin (NTP 1990, 1992 - no robust summaries prepared) delivered in corn oil by gavage are consistently associated with these phenomena in the forestomach of rodents. Squamous cell papillomas are benign lesion of surfaces covered with squamous epithelium. A majority of papillomas arise as a result of chronic irritation, or from infection from some strains of viruses (Smith and Ford, 1993 - no robust summary prepared). Additionally, forestomach hyperplasia and papillary proliferation in these studies did not progress to squamous cell carcinomas.

Apparently, the combination of daily introduction of a dosing needle into the forestomach and delivery of a high concentrations of an irritating test material in corn oil, which itself is a mild irritant and mitogen, was the likely source of the papillomas in the rodent forestomach. This conclusion is supported by the observation that the occurrence of squamous cell papillomas and forestomach hyperplasia in gavage administration of a test material in corn oil for 2 years (see below; NTP, 1986 - no robust summary prepared) disappear when the same substance is administered at similar intake levels in the diet (NTP, 1993 - no robust summary prepared). Therefore, the appearance of these benign lesions in the 2-year rodent bioassay have no relevance to humans, given that human exposure occurs when low levels of benzaldehyde are consumed in the diet.

In conclusion, evidence of carcinogenicity in the gavage benzaldehyde study is associated with the repeated gavage administration of high dose levels of test substance in a corn oil vehicle or a statistical anomaly. The above observations strongly suggest that the results of the gavage NTP studies have no significance to humans.

This conclusion is further supported by the lack of tumorigenicity in a chronic studies conducted with a structural representative of this group, sodium benzoate, and the primary metabolite, benzoic acid. No effect on tumor incidence or survival was seen in male and female albino Swiss mice administered 2% sodium benzoate in drinking water (approximately 4,000 mg/kg bw/day) for their life span (up to approximately 112 weeks) [Toth, 1984]. The only reported finding in a 17-month study in which mice were orally administered 40 mg/kg bw/day of benzoic acid was increased survival as compared to controls [Shtenberg and Ignat'ev, 1970].

In a standard cancer bioassay B6C3F1 mice were fed up to 5000 ppm dimethyl terephthalate in the diet for 2 years with no adverse effects with the exception of a statistically significant increase in lung tumors in treated male mice [Federal Register, 1981 – no robust summary prepared]. The authors reported that the increased lung tumor incidence in male mice has been peer reviewed a few times and it was finally concluded that this finding was considered biologically equivocal due to the lack of similar findings in female mice and an exceptionally low incidence of lung tumors in control males.

3.4.4 Reproductive Toxicity

Studies examining possible reproductive toxicity are available on the principal metabolite (terephthalic acid), dimethyl terephthalate and benzaldehyde.

Male rats had been fed 0.25, 0.50, or 1.0% dimethyl terephthalate in the diet for 115 days were mated with females, which had been fed the same diets for 6 days [Krasavage *et al.*, 1973]. Females were maintained on the treated diets throughout gestation, parturition, and lactation. No effects on fertility, reproductive capacity, libido, pregnancy, gestation, litter size, or pup viability were reported. Significantly lower average pup body weights at weaning were reported in offspring of rats fed 0.5 or 1.0% dimethyl terephthalate

compared to controls. The authors suggested that the decreased pup body weight was related to lactation exposure to dimethyl terephthalate or its metabolite terephthalic acid plus access to treated diet.

In a GLP-compliant study, 2 strains of rats (Wistar and CD) were compared in a 1-generation study testing terephthalic acid fed at dietary concentrations up to 5% (approximately 2500-3000 mg/kg bw/day) [Chemical Industry Institute of Technology (CIIT), 1982]. Parental rats were fed terephthalic acid 90 days prior to mating and throughout mating. Maternal exposure continued throughout gestation and lactation and offspring exposure continued to 30 days post-weaning. Parameters evaluated included fertility index, number of offspring born/dam, number and proportion of each sex born, number (Day 0, 1, and 21) and proportion (Day 1 and 21) of each sex alive, and average weight at Day 1 and 21 of all offspring and of each sex. At study termination, all offspring were killed, grossly examined and necropsied. Body weights were significantly decreased after 13 weeks at 2 and 5% in both sexes of CD rats, at 0.03% in CD males and at 5% in Wistar rats of both sexes. At 5%, death occurred in 3 CD female and 1 Wistar rat/sex. During the one-generation component of the study 3 CD rats (1 male at 2% and 1/sex at 5%) and 4 female Wistar rats (2 at 5% and 2 at 0.03%) died. No effect on fertility index or litter size was reported. No effects on litter size, sex ratio, or total number of offspring were reported. On Day 0, 17 Wistar offspring (1 at 0.03%, 2 at 0.125%, 1 at 0.5%, 12 at 2% [2 from 1 dam, 10 from another]) and 23 CD offspring (1 at 0.5%, 7 at 2% [3 dams] and 15 at 5% [3 dams; with 11 from 1 of them]) were found dead. No statistical differences were reported in viability of either strain on Days 0, 1, or 21. Survival on Day 21 of CD offspring of rats fed 5% was reduced. On Day 1, body weights were reduced in Wistar rats at 5%. On Day 21, body weights were reduced in both rat strains at 5%. At these levels, several pups were not allowed to nurse by dams showing signs of toxicity and several large litters were lost. During the post weaning period (Day 21-51), 18 Wistar and 16 CD rats at 5% died. A high incidence of renal and bladder calculi were reported in these animals. Renal and bladder calculi also were reported in all necropsied rats fed 5% terephthalic acid. The parental NOAEL was reported to be 0.5 and 2.0% for CD and Wistar rats, respectively. The reproductive NOAEL for both strains was greater than 5.0%. The F1 offspring NOAEL was reported to be 0.5% for both strains.

Approximately 5 mg/kg bw/day of benzaldehyde was administered by gavage to 10 breeding age rats every other day for a period of 32 weeks [Sporn *et al.*, 1967]. Two pregnancies per rat were studied, one at 75 days and one at 180 days. There was no statistical significant difference between treatment and control groups. It was reported that fewer females in the treated group became pregnant; however, no data or statistical analyses were performed, and the authors concluded that treatment did not cause a significant change in any of the reproductive parameters measured.

3.4.5 Developmental Toxicity

Developmental toxicity has been tested in terephthalic acid, the *in vivo* metabolite of methyl 4-formylbenzoate. Also included are data for dimethyl terephthalate, a substance that also forms terephthalic acid *in vivo*, and benzyl alcohol, a metabolic precursor of the mono-functional analog benzaldehyde.

Overall, the substances from this group were tested for developmental toxicity with uniform results that indicate no teratogenic potential in the absence of maternal toxicity.

3.4.5.1 Rats

Pregnant Wistar rats were gavaged with 1000 mg/kg bw/day of dimethyl terephthalate throughout gestation Days 7-16 and killed on Day 21 [Hoechst, 1986]. There were no signs of maternal toxicity and no abnormal developmental effects and no pre- or post-implantation losses were reported.

In a GLP-compliant study, pregnant rats underwent whole body inhalation exposures of 1.0, 5.0, or 10.0 mg/m³, 6 hours/day of terephthalic acid throughout gestation Days 6-15 [Amoco Corporation, 1989b]. Rats were killed on Day 21 and "standard" guideline postmortem exams were conducted on dams and fetuses. No maternal deaths were reported and there were no significant differences in clinical signs, mean body weight or weight gain, uterine weight or implant number compared to controls. No statistically significant differences from control were reported in mean litter weights, pup viability, or number of fetal malformations. There was no difference from control noted in external

soft tissue examination; however, there was a slight increase in the incidence of rib anomalies (all types added together) at 5.0 mg/m³. The rib anomalies reported at the middle dose were not considered to be an indicator of teratogenicity since they are a common variation, were not dose-dependent, were not accompanied by other signs of embryotoxicity, and were within the range of historical controls for the laboratory.

3.4.5.2 Mice

In a teratology study, groups of pregnant CD-1 mice were administered 0 or 550 mg/kg bw/day of benzyl alcohol in corn oil by gavage during gestation Days 6-15 [York *et al.*, 1986]. Body weight, clinical observations, and mortality were recorded daily throughout treatment and up to 3 days postpartum. All parameters tested, including gestation index, average number of live pups/litter, postnatal survival, and pup body weight, were statistically similar for the treated and control animals.

Groups of CD-1 mice were gavaged with 750 mg/kg bw/day of benzyl alcohol on gestation Days 6-13 [Hardin *et al.*, 1987]. Controls received distilled water only. Clinical signs of maternal toxicity were reported and included hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnea, swollen or cyanotic abdomen, and piloerection. There was no significant difference in maternal body weight measured on Days 4 and 7 of gestation between treated and control animals; however, statistically significant decreases were observed in treated females on gestation Day 18 and Day 3 postpartum. Maternal body weight gain during Days 7-18 of gestation was also significantly lower than that of controls. Significant differences were also observed in pup body weight and weight gain, including mean pup weight per litter, mean litter weight change, and mean pup weight change (between Day 1 and 3 postpartum). No differences were observed in the mating or gestation indices, the total number of resorptions, the number of live pups per litter, or in pup survival. Eighteen deaths were reported during the treatment period and they were all attributed to the treatment. One more death was reported the day after treatment was terminated. Although the authors concluded that benzyl alcohol was a potential reproductive hazard, the effects observed were in conjunction with significant maternal toxicity.

3.4.6 New Testing Required

Numerous genetic, acute, repeat-dose, developmental, and reproductive toxicity studies exist for the principal metabolite, metabolic precursors of that metabolite, and other structurally related mono-functional analogs. Toxicological data on these substances are directly related to the evaluation of methyl 4-formylbenzoate. Moreover, the dose levels tested provide adequate margins of safety to accommodate any differences in structure between methyl 4-formylbenzoate and these substances.

The only significant effect in any of these studies is the development of renal calculi and renal tumors in a chronic rat study. This phenomenon is associated with the renal processing of extremely high dose levels of organic acids (*i.e.*, terephthalic acid). Chronic exposure to such acids leads to cumulative formation of renal calculi composed principally of calcium salts of these acids. Accumulation of calculi in the kidney leads to renal hyperplasia progressing eventually to tumors. These effects would not be observed in humans under normal conditions of exposure. In other studies at lower dose levels there is no evidence of the formation of renal calculi, renal hyperplasia or tumor formation.

3.5 TEST PLAN TABLE

Chemical	Physical-Chemical Properties					
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility	
CAS No. 001571-08-0 Methyl 4-formylbenzoate	A	A	Calc	Calc	Calc	
Chemical	Environmental Fate and Pathways					
	Photodegradation		Stability in Water	Biodegradation	Fugacity	
CAS No. 001571-08-0 Methyl 4-formylbenzoate	Calc		R, Calc	R, Calc	Calc	
Chemical	Ecotoxicity					
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates		Acute Toxicity to Aquatic Plants		
CAS No. 001571-08-0 Methyl 4-formylbenzoate	R, Calc	R, Calc		R, Calc		
Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity <i>In Vitro</i>	Genetic Toxicity <i>In Vivo</i>	Repeat Dose Toxicity	Reproductive Toxicity	Developmental Toxicity
CAS No. 001571-08-0 Methyl 4-formylbenzoate	A	R	R	R	R	R

Legend	
Symbol	Description
R	Endpoint requirement fulfilled using category approach, SAR
T	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

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