

## SCREENING-LEVEL HAZARD CHARACTERIZATION

### **N-oxydiethylenebenzothiazole-2-sulfenamide (CASRN 102-77-2)**

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

The Environmental Protection Agency’s Office of Pollution Prevention and Toxics (OPPT) is evaluating the data submitted in the HPV Challenge Program on approximately 1400 sponsored chemicals by developing hazard characterizations (HCs). These HCs consist of an evaluation of the quality and completeness of the data set provided in the Challenge Program submissions. They are not intended to be definitive statements regarding the possibility of unreasonable risk of injury to health or the environment.

The evaluation is performed according to established EPA guidance<sup>2,3</sup> and is based primarily on hazard data provided by sponsors; however, in preparing the hazard characterization, EPA considered its own comments and public comments on the original submission as well as the sponsor’s responses to comments and revisions made to the submission. In order to determine whether any new hazard information was developed since the time of the HPV submission, a search of the following databases was made from one year prior to the date of the HPV Challenge submission to the present: (ChemID to locate available data sources including Medline/PubMed, Toxline, HSDB, IRIS, NTP, ATSDR, IARC, EXTOXNET, EPA SRS, etc.), STN/CAS online databases (Registry file for locators, ChemAbs for toxicology data, RTECS, Merck, etc.) and Science Direct. OPPT’s focus on these specific sources is based on their being of high quality, highly relevant to hazard characterization, and publicly available.

OPPT does not develop HCs for those HPV chemicals which have already been assessed internationally through the HPV program of the Organization for Economic Cooperation and Development (OECD) and for which Screening Initial Data Set (SIDS) Initial Assessment Reports (SIAR) and SIDS Initial Assessment Profiles (SIAP) are available. These documents are presented in an international forum that involves review and endorsement by governmental authorities around the world. OPPT is an active participant in these meetings and accepts these documents as reliable screening-level hazard assessments.

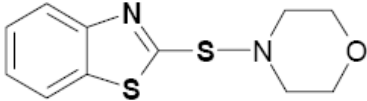
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<sup>1</sup> U.S. EPA. High Production Volume (HPV) Challenge Program; <http://www.epa.gov/chemrtk/index.htm>.

<sup>2</sup> U.S. EPA. HPV Challenge Program – Information Sources; <http://www.epa.gov/chemrtk/pubs/general/guidocs.htm>.

<sup>3</sup> U.S. EPA. Risk Assessment Guidelines; <http://cfpub.epa.gov/ncea/raf/rafguid.cfm>.

These hazard characterizations are technical documents intended to inform subsequent decisions and actions by OPPT. Accordingly, the documents are not written with the goal of informing the general public. However, they do provide a vehicle for public access to a concise assessment of the raw technical data on HPV chemicals and provide information previously not readily available to the public.

<p><b>Chemical Abstract Service Registry Number (CASRN)</b></p>	<p><b>102-77-2</b></p>
<p><b>Chemical Abstract Index Name</b></p>	<p><b>Benzothiazole, 2-(4-morpholinylthio)-</b></p>
<p><b>Structural Formula</b></p>	
<p style="text-align: center;"><b>Summary</b></p> <p>CASRN 102-77-2 is a solid at room temperature with low vapor pressure. The water solubility of CASRN 102-77-2 cannot be accurately determined because it undergoes hydrolysis in water within the timescale of the solubility experiment. Hydrolysis is the key determinant of the environmental fate of CASRN 102-77-2. It is expected to have moderate mobility in soil. Volatilization of CASRN 102-77-2 is considered negligible based on its Henry's Law constant. The rate of hydrolysis is considered moderate to rapid. The rate of atmospheric photooxidation is considered rapid. Due to this instability in water, CASRN 102-77-2 is expected to have low persistence (P1) and low bioaccumulation potential (B1).</p> <p>The acute toxicity of CASRN 102-77-2 is low in both rats (oral and inhalation routes of exposure) and rabbits (dermal route of exposure). In a two-year oral repeated-dose study with rats (dietary exposure), there were no adverse effects up to the highest dose of CASRN 102-77-2 tested. The NOAEL for systemic toxicity is 400 mg/kg/day (highest dose tested). A 21-day dermal study in rabbits showed changes in hematology measures at a dose of 2000 mg/kg/day CASRN 102-77-2; the NOAEL for systemic toxicity is 500 mg/kg/day. In a 28-day inhalation study with rats, there were no adverse effects observed up to the highest tested concentration of CASRN 102-77-2; the NOAEC for systemic toxicity is 0.0102 mg/L (highest concentration tested). There is no specific reproductive toxicity study available for CASRN 102-77-2; however, this endpoint has been satisfied for the purposes of the HPV Challenge Program because there is a developmental toxicity study (no developmental effects), the reproductive organs were evaluated in the two-year repeated-dose study (no effects observed), and there was a dominant lethal test in male rats which showed no effects up to the highest dose tested (500 mg/kg/day via gavage for 56 days). In the prenatal developmental toxicity study with CASRN 102-77-2 (in rats, via gavage), there was maternal toxicity observed at 300 mg/kg/day (decreased body weight gains and clinical signs); the NOAEL for maternal toxicity is 100 mg/kg/day. There was no developmental toxicity observed up to the highest tested dose; the NOAEL for developmental toxicity is 1000 mg/kg/day. CASRN 102-77-2 was negative for gene mutations when tested in bacteria <i>in vitro</i> but positive (with metabolic activation) when tested in mammalian cells <i>in vitro</i>. CASRN 102-77-2 did not induce chromosomal aberrations in mammalian cells <i>in vitro</i>. CASRN 102-77-2 was positive in a DNA repair assay in bacteria and equivocal in sister chromatid exchange studies in mammalian cells <i>in vitro</i>. CASRN 102-77-2 is irritating to the skin and eye in rabbits and is a skin sensitizer in humans. CASRN 102-77-2 was not carcinogenic in a two-year rat study.</p>	

The acute toxicity data for fish exposed to CASRN 102-77-2 ranges from 0.31 to 3.5 mg/L. The acute toxicity data for aquatic invertebrates exposed to CASRN 102-77-2 is 4.0 mg/L. The acute toxicity to aquatic plants from exposure to CASRN 102-77-2 is 2.0 mg/L (biomass/growth).

No data gaps were identified under the HPV Challenge Program.

The sponsor, The Rubber and Plastic Additives Panel (RAPA Panel) of the American Chemistry Council, submitted a Test Plan and Robust Summaries to EPA for the category Sulfenamide Accelerators on November 30, 2001. The proposed category consisted of two sponsored chemicals (CASRN 102-77-2 and 13752-51-7). EPA posted the submission on the ChemRTK HPV Challenge Website on December 20, 2001. EPA comments were posted to the HPV Challenge Web site on August 20, 2002. Public comments were also received and posted to the website. Based on the EPA and other comments, the RAPA Panel revised the category submission by submitting two separate, single chemical submissions. New test plans and robust summaries for N-oxydiethylenebenzothiazole-2-sulfenamide, (CASRN 102-77-2), were submitted on August 25, 2003. EPA posted the submission on the ChemRTK HPV Challenge Web site on October 24, 2003 (<http://www.epa.gov/chemrtk/pubs/summaries/sulfaccl/c13323tc.htm>). Subsequent submissions by the sponsor pertain to the second chemical of the initial category (N-oxydiethylenethiocarbamoyl-N'-oxydiethylenesulfenamide, CASRN 13752-51-7) – for which a Risk-Based Prioritization Document has been posted (see [http://www.epa.gov/chemrtk/hpvis/rbp/13752-51-7\\_N-oxydiethylenethiocarbamyl\\_Web\\_April%202009.pdf](http://www.epa.gov/chemrtk/hpvis/rbp/13752-51-7_N-oxydiethylenethiocarbamyl_Web_April%202009.pdf)).

## **1. Chemical Identity**

### **1.1 Identification and Purity**

CASRN 102-77-2 is benzothiazole, 2-(4-morpholinylthio) - , also known commercially as Santocure MOR Accelerator. The various robust summaries indicate that a purity of 95-99% is typical for commercial products.

### **1.2 Physical-Chemical Properties**

The physical-chemical properties of CASRN 102-77-2 are summarized in Table 1.

CASRN 102-77-2 is an off-white tan solid with an indeterminate water solubility and low vapor pressure. Measured physical-chemical properties may be more reflective of the hydrolysis products of benzothiazole, 2-(4-morpholinylthio)-. The hydrolysis products reported by the sponsor are 64% benzothiazole (CASRN 95-16-9), 21% 2-mercaptobenzothiazole (CASRN 149-30-4), 15% an unknown proposed compound  $C_{11}H_{14}S_2N_2O_2$ , and an undetermined amount of morpholine (CASRN 110-91-8).

<b>Property</b>	<b>Value</b>
CASRN	102-77-2
Molecular Weight	252.35
Physical State	Solid, off-white to tan
Melting Point	82-88°C (measured)
Boiling Point	Decomposition begins at 216.1°C and is complete at 219.37 °C
Vapor Pressure	$5.1 \times 10^{-6}$ mm Hg at 25°C (estimated) <sup>3</sup>
Water Solubility	5,848 mg/L at 25°C (estimated) <sup>3</sup> ; 0.039 mg/L at 25°C (measured, degradation noted) <sup>4</sup>
Dissociation Constant (pK <sub>a</sub> )	Not applicable
Henry's Law Constant	$<1.0 \times 10^{-10}$ atm·m <sup>3</sup> /mole (estimated) <sup>3</sup>
Log K <sub>ow</sub>	3.49 (measured) <sup>5</sup> ; 1.02 (estimated) <sup>3</sup>

<sup>1</sup> Rubber and Plastic Additives Panel, American Chemistry Council August 27, 2003. Revised Robust Summary and Test Plan for 2-(Morpholinothio)benzothiazole. Available online from: <http://www.epa.gov/chemrtk/pubs/summaries/sulfaccl/c13323tc.htm> or <http://www.epa.gov/chemrtk/pubs/summaries/bnzhict/c13324tc.htm> as of May 20, 2010.

<sup>2</sup> The measured data submitted by the sponsor was generated from a test substance (Santocure MOR) containing 95-99% benzothiazole, 2-(4-morpholinylthio)-, with typical impurities being <0.4% morpholine (CASRN 110-91-8), <1% 2-mercaptobenzothiazole (CASRN 149-30-4), <1% mercaptobenzothiazole disulfide (CASRN 120-78-5), and <0.4% benzothiazole (CASRN 95-16-9).

<sup>3</sup> U.S. EPA. 2010. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.00. U.S. Environmental Protection Agency, Washington, DC, USA. Available online from: <http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm> as of May 20, 2010.

<sup>4</sup> The water solubility reported in the Robust Summary, does not agree well with the estimated value for this test compound. The measured value of 0.039 mg/L noted that the compound was unstable under the test conditions and degradation occurred during the test. These degradation products were not identified or quantified. Benzothiazole, 2-(4-morpholinylthio) - hydrolyzes rapidly in water and consequently the measured value is lower than expected for this compound and represents only the unhydrolyzed material. The experimental details note that the sample was stirred in the dark for several days.

<sup>5</sup> The log K<sub>ow</sub> reported in the Robust Summary, does not agree well with the estimated value for this test compound. The measured value of 3.49 is provided for the commercial product, Santocure MOR accelerator, which may contain impurities or undergo hydrolysis during the n-octanol/water partitioning experiment. The experimental details note that the sample was shaken for 48 hours followed by equilibration for several days prior to the measurement

## **2. General Information on Exposure**

### **2.1 Production Volume and Use Pattern**

CASRN 102-77-2 had an aggregated production and/or import volume in the United States between 1 and 10 million pounds during calendar year 2005.

Non-confidential information in the IUR indicated that the industrial processing and uses of the chemical include resin and synthetic rubber manufacturing, tire manufacturing, and other rubber product manufacturing as process regulators, used in vulcanization or polymerization processes.

Non-confidential commercial and consumer uses of this chemical include not readily obtainable (NRO).

## 2.2 Environmental Exposure and Fate

Table 2 lists the environmental fate properties of CASRN 102-77-2.

CASRN 102-77-2 is expected to have moderate mobility in soil. CASRN 102-77-2 was not readily biodegradable, (0% degradation in 28 days), using a modified MITI test under directive 84/449/EEC, C.7 - Biotic Degradation. CASRN 102-77-2 was completely hydrolyzed within 7 days at pH 7; however, no data were presented regarding the hydrolysis rates under acidic or alkaline conditions. Therefore, the rate of hydrolysis is expected to be moderate to rapid under environmental pH and temperature. The rate of volatilization is considered negligible based on its Henry's Law constant. CASRN 102-77-2 is expected to have low persistence (P1) and low bioaccumulation potential (B1).

<b>Property</b>	<b>Value</b>
Photodegradation Half-life	1.1 hours (estimated) <sup>2</sup> 1.0 hours (measured, direct photolysis)
Hydrolysis Half-life	24% degradation after 25 hours at pH 7 and 20°C (measured), 100% degradation after 7 days at pH 7 at 20°C (measured) <sup>3</sup>
Biodegradation	0% biodegradation in 28 days (not readily biodegradable)
Bioaccumulation Factor	BAF =1.5 (estimated) <sup>2</sup>
Log K <sub>oc</sub>	3.4 (estimated) <sup>2</sup>
Fugacity (Level III Model) <sup>2</sup>	
Air (%)	<0.1
Water (%)	11.2
Soil (%)	87.1
Sediment (%)	1.7
Persistence <sup>4</sup>	P1 (low)
Bioaccumulation <sup>4</sup>	B1(low)

<sup>1</sup>Rubber and Plastic Additives Panel, American Chemistry Council August 27, 2003. Revised Robust Summary and Test Plan for 2-(Morpholiniothio)benzothiazole. Available online from:

<http://www.epa.gov/chemrtk/pubs/summaries/sulfaccl/c13323tc.htm> or

<http://www.epa.gov/chemrtk/pubs/summaries/bnzthict/c13324tc.htm> as of May 20, 2010.

<sup>2</sup>U.S. EPA. 2010. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.00. U.S. Environmental Protection Agency, Washington, DC, USA. Available online from:

<http://www.epa.gov/opptintr/exposure/pubs/episuitedi.htm> as of April 1, 2010

<sup>3</sup>Hydrolysis products and percentages identified and quantified by HPLC and GC/MS were reported by the sponsor as benzothiazole (CASRN 95-16-9) 64%, 2-mercaptobenzothiazole (CASRN 149-30-4) 21%, an unknown proposed compound C<sub>11</sub>H<sub>14</sub>S<sub>2</sub>N<sub>2</sub>O<sub>2</sub>, 15%, and morpholine (CASRN 110-91-8), % not determined from a study cited as Monsanto AB-84-131, Analytical Bio-Chemistry Labs, 1984.

<sup>4</sup>Federal Register. 1999. Category for Persistent, Bioaccumulative, and Toxic New Chemical Substances. *Federal Register* 64, Number 213 (November 4, 1999) pp. 60194–60204.

**Conclusions:** CASRN 102-77-2 is a solid at room temperature with low vapor pressure. The water solubility of CASRN 102-77-2 cannot be accurately determined because it undergoes hydrolysis in water within the timescale of the solubility experiment. Hydrolysis is the key determinant of the environmental fate of CASRN 102-77-2. It is expected to have moderate mobility in soil. Volatilization of CASRN 102-77-2 is considered negligible based on its Henry's Law constant. The rate of hydrolysis is considered moderate to rapid. The rate of atmospheric photooxidation is considered rapid. Due to this instability in water, CASRN 102-77-2 is expected to have low persistence (P1) and low bioaccumulation potential (B1).

### 3. Human Health Hazard

A summary of health effects data submitted for SIDS endpoints is provided in Table 3.

#### *Acute Oral Toxicity*

(1) Wistar rats (10/sex) were administered CASRN 102-77-2 via gavage at 10,000 mg/kg. No mortalities were reported.

**LD<sub>50</sub> > 10,000 mg/kg**

(2) Sprague-Dawley albino rats (2-3/ sex/dose) were administered CASRN 102-77-2 via gavage at 5010, 6310 or 7940mg/kg. There was a single death (one female) in the high dose group only.

**LD<sub>50</sub> > 7940 mg/kg**

#### *Acute Inhalation Toxicity*

Albino rats (20 animals; male and female; 10 treated and 10 controls; strain not specified) were exposed to CASRN 102-77-2 as undiluted powder at a single concentration 0.09 mg/L for four hours. There were no mortalities.

**LC<sub>50</sub> > 0.09 mg/L**

#### *Acute Dermal Toxicity*

New Zealand rabbits (four animals total; two/sex) were exposed to CASRN 102-77-2 via the dermal route at doses of 3160 (one female), 5010 (one male) or 7940 (one male and one female) mg/kg. The test material was applied to shaved skin which was occluded for 24 hours. No mortality was reported

**LD<sub>50</sub> > 5010 mg/kg**

#### *Repeated-Dose Toxicity*

##### *Oral*

(1) Sprague-Dawley rats (5/sex/treatment) were exposed to CASRN 102-77-2 via the diet at 0, 100, 200, 500 or 1000 mg/kg/day for 4-weeks. This was a range-finding study for a teratology study to determine both the stability of the chemical in the diet and its palatability to rats. The only parameters evaluated were clinical signs, body weight and food consumption, organ weight, and gross examination of organs at necropsy (there were no blood, urine or histopathological

analyses). There were no mortalities. Male rats at the two highest doses gained weight at a reduced pace compared to controls beginning at the first week of treatment, resulting in a 12% decrease (not stated if this was for either the 500 or 1000 mg/kg/day groups, or both). Mean absolute liver and relative kidney weights in high-dose males were higher than controls (incidence or severity not stated).

(2) Sprague-Dawley rats (50/sex/treatment) were exposed to CASRN 102-77-2 through the diet at doses of 0, 5, 50, or 400 mg/kg/day for 2 years. Parameters evaluated included body weight, food consumption, behavior, hematology, clinical chemistry, urinalysis, major organ assessment (weight, gross lesions, and histological analysis in control, mid- and high-dose animals). There was no mortality attributed to exposure to the test material. All animals at 50 and 400 mg/kg/day experienced statistically significant reductions in both body weight gain and food consumption as compared to controls; however, there was no apparent decrease in overall body weight. Increases in kidney and liver weights were also observed at 50 and 400 mg/kg/day (incidence, magnitude and statistical significance not stated). However, there were no pathological findings reported for these or any other organ.

**NOAEL = 400 mg/kg/day (highest tested dose)**

### ***Dermal***

New Zealand rabbits (10 animals/sex/treatment) were exposed to CASRN 102-77-2 via the dermal route at 0, 125, 500 or 2000 mg/kg/day for 21-days (6-hrs/day, 5d/wk). Half of the treatment animals were exposed with skin intact and half with abraded skin. The following parameters were evaluated: clinical signs, body weight, dermal irritation, blood and urine analyses, organ weights, and macroscopic and some microscopic organ evaluations. One death occurred in each treated group and one animal from the control group was euthanized *in extremis*; all deaths were considered to be not treatment-related. Clinical signs that were observed were not treatment-related as they were noted in all groups (nasal and eye discharge, soft stool and possible anorexia). There were no statistically significant changes in mean body weights. Although not specifically stated in the summary, there appeared to be no treatment-related effects on organ weights and no macro- or microscopic alterations in any organs. A few rabbits in the treatment groups exhibited very slight to slight erythema, edema and desquamation. Similar findings (slight desquamation and red raised areas) were reported in a few animals from the control group. Other lesions on the skin at the application site of all (control and treated) animals were hyperkeratosis and infiltration of inflammatory cells in the dermis. Differences reported between control and treated animals were decreases in hematocrit, hemoglobin and total erythrocytes in females and lower lactate dehydrogenase (LDH) and higher cholesterol in males at 2000 mg/kg/day.

**LOAEL = 2000 mg/kg/day (based on changes in several hematological parameters)**

**NOAEL = 500 mg/kg/day**

### ***Inhalation***

Sprague-Dawley rats (5 animals/ sex/ dose) were exposed to CASRN 102-77-2 via inhalation as a fine dust at concentrations of 0, 4.4, 9.8 or 10.2 mg/m<sup>3</sup> (0, 0.004, 0.0098 or 0.0102 mg/L) for 6 hours/day for 5 days/ week for 4-weeks. All animals survived study duration. Exposure to the test article caused slight irritation in exposed animals. Slight body weight reductions and reductions in lung weights were observed in male rats in the high concentration group (0.0102 mg/L). No other organ weight or histopathological changes were reported. There were slight

depressions in blood glucose and elevations in serum glutamate-oxaloacetate transaminase (SGOT) in rats at all exposure levels. The summary did not provide incidence, severity, or level of statistical significance for any of the effects noted.

**NOAEC = 0.0102 mg/L (highest tested concentration)**

### ***Reproductive Toxicity***

There was no conventional reproductive toxicity study performed with CASRN 102-77-2; however, this endpoint has been satisfied for the purposes of the HPV Challenge Program because there is a developmental toxicity study (no developmental effects, see below), the reproductive organs were evaluated (with no effects observed) in the two-year study described earlier, and there was a dominant lethal test in male rats which is summarized in the Genetic Toxicity (Other) section below.

### ***Developmental Toxicity***

Developmental toxicity was evaluated in Sprague-Dawley rats (25 animals/dose) exposed to CASRN 102-77-2 via gavage at 0, 100, 300 or 1000 mg/kg/day daily during days 6-15 of gestation. Caesarean sections were performed on all dams on day-20 of gestation. One mortality was reported in the high-dose group on gestation day-14 and was attributed to gavage error. Maternal toxicity was observed as a dose-related increase in matting and staining of the anogenital hair coat and a decrease in mean maternal body weight gain; both in the 300 and 1000 mg/kg/day treatment groups. Although no data are provided in the summary, the submitter states that there were “no biologically meaningful or statistically significant differences” in any of the following pregnancy/litter parameters: mean numbers or corpora lutea, total implantations, viable fetuses, early or late resorptions, postimplantation loss, mean fetal body weights or mean fetal sex distribution, or mean number of litters with malformations, developmental or genetic variations.

**LOAEL (maternal toxicity) = 300 mg/kg/day (based on decreased maternal body weight gains and clinical signs)**

**NOAEL (maternal toxicity) = 100 mg/kg/day**

**NOAEL (developmental toxicity) = 1000 mg/kg/day (highest tested dose)**

### ***Genetic Toxicity – Gene Mutation***

#### ***In vitro***

(1) In four separate bacterial reverse mutation tests, either *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 strains (four tests) or *Escherichia Coli* strain WP2uvrA (one test) were exposed to CASRN 102-77-2 with and without metabolic activation. The test concentrations ranged between 0.1 and 5000 µg/plate. Cytotoxicity was reported at concentration ranges of 500 to 1000 µg/plate and above. The test compound did not demonstrate mutagenic activity in any of the assays conducted and was considered non-mutagenic under the test conditions. Positive and solvent controls were conducted and resulted in the appropriate outcome.

**CASRN 102-77-2 was not mutagenic in this assay.**

(2) In two separate mammalian cell forward gene mutation assays with mouse lymphoma cells L5178Y TK+/- with and without metabolic activation CASRN 102-77-2 was used at concentration ranges of 0.156 to 15.0 µg/ ml without metabolic activation and 1.56 to 50 µg/ ml with activation. Cytotoxicity was reported at 40 µg/ ml and higher (with activation) and 12.5 µg/ ml and higher (without metabolic activation). In both experiments, the test material induced significant increases in the TK locus in L5178Y mouse lymphoma cells only in the presence of S9 activation and generally at high (near-toxic) concentrations. In the absence of microsomal activation, moderately toxic concentrations up to 7.5 ug/ml were not mutagenic. The test compound was considered to be active only in the presence of activation in these assays. **CASRN 102-77-2 was mutagenic in this assay (with metabolic activation only).**

### ***Genetic Toxicity – Chromosomal Aberrations***

#### ***In vitro***

A chromosomal aberration study with CASRN 102-77-2 was reported in Hinderer et al., 1983. Chinese Hamster Ovary (CHO) cells were used and CASRN 102-77-2 was tested at concentrations of 0.625 to 10 ug/ml (both with and without activation). Appropriate positive and negative controls were used. Results showed no increase in the frequency of aberrations/cell. **CASRN 102-77-2 did not induce chromosomal aberrations in this study.**

#### ***Genetic Toxicity – Other***

(1) An *in vitro* DNA Repair Test was conducted using *Escherichia coli* W3110 (*pol* A+) and W3078 (*pol* A-), with and without metabolic activation with concentrations of CASRN 102-77-2 ranging from 0.5-2500 µg/plate. Cytotoxicity was not determined. Positive and negative (solvent) controls were run concurrently; the appropriate result was achieved with the positive control. A survival index of 0.85 or less was considered positive. The summary noted “some slight reductions in the survival index”; however, a dose response was not apparent. There were no specifics provided (i.e., survival index by concentration). EPA obtained the published article (Hinderer et al., 1983) which contains all the data showing both a dose-response and an apparent effect (Table V in cited article). The study authors concluded that CASRN 102-77-2 was mutagenic in this assay.

**CASRN 102-77-2 was genotoxic in this assay.**

(2) Four Sister Chromatid Exchange (SCE) studies with CASRN 102-77-2 were reported using Chinese Hamster Ovary (CHO) cells. The ranges of concentrations used were 0.625 to 80µg/ml (w/o metabolic activation) and 0.625 to 240 (with metabolic activation). Cytotoxicity occurred at 100 µg/ ml (with activation) and 50 µg/ ml (without activation). Positive and negative (solvent) controls were run concomitantly and the appropriate results were achieved. Statistically significant (t-test,  $p \leq 0.05$ ) but less than 2-fold increase in SCE frequency was observed at the high doses of two experiments without metabolic activation and at a range of concentrations with metabolic activation. The summaries describing the remaining two experiments reported negative results.

**CASRN 102-77-2 yielded equivocal results which will be considered positive for genotoxicity.**

(3) Male-mediated genotoxicity effects were evaluated in rats (10/dose level) in a dominant lethal test in which CASRN 102-77-2 was administered via gavage at dose levels of 0, 125, 250 or 500 mg/kg/day for 56 consecutive days. The males were then housed with non-treated females for two weeks and allowed to mate. Thirteen days after mating the females were sacrificed and evaluated for embryo lethality. Treatment had no adverse effects on the males with respect to clinical signs, mortality rate, and body weight gain or organ weights. No evidence of reduced fertility was observed. No effects were reported on pregnancy, early fetal death, implantation or preimplantation losses in females. A positive control was used and resulted in the appropriate outcome.

**NOAEL (dominant-lethal toxicity-males) = 500 mg/kg/day (highest tested dose)**

### *Additional Information*

#### *Skin Irritation*

New Zealand rabbits (six, gender not specified) were exposed to 0.5 grams of CASRN 102-77-2 as a fine powder to their shaved backs. The area was covered with a gauze patch for 24 hours, removed, and the skin was scored for irritation using the Draize method at 24, 48, 72 and 168 hours. The Primary Irritation Index (PII) was 0.6 (average of the mean scores at 24 and 72 hours).

**CASRN 102-77-2 was slightly irritating to the skin.**

#### *Eye Irritation*

New Zealand rabbits (six, gender not specified) were exposed to 100 mg of CASRN 102-77-2 as a fine powder to one eye (the other eye was not treated and served as the control). The eyes were examined immediately after 24 hours of treatment and then 10 minutes, and one, 24, 48, 72 and 168 hours later. Irritation was noted at 24 hours and then was reduced at 48 and 72 hours and resolved to no irritation by 168 hours.

**CASRN 102-77-2 was slightly irritating to the eye.**

#### *Sensitization*

(1) Forty-nine human volunteers (gender and age distribution not reported) participated in a repeated-insult patch test with CASRN 102-77-2 (96% pure, 75% preparation in petrolatum). The robust summary stated that the 1953 Shelanski-Shelanski method was used. Although not described in the summary, this method calls for volunteers to wear patches on the upper arm three times a week for 24 hours each time for a 2-3 week period (induction period). After a 2-3 week rest period, the volunteers are challenged with a patch for 48 hours. Both irritation and sensitization reactions are scored. There was no evidence of primary irritation and 49% (24/49) people had a sensitization reaction.

**CASRN 102-77-2 was considered a human sensitizer in this test.**

(2) Fifty-one human volunteers (gender and age distribution not reported) participated in a repeated-insult patch test with CASRN 102-77-2 (96% pure, 30 subjects were exposed to 10% preparation and 21 subjects were exposed to a 1% preparation; both in petrolatum). The robust

summary stated that the 1953 Shelanski-Shelanski method was used. As described in the summary, the induction phase exposures were four days/week (duration not stated) for three weeks followed by a one week rest period and a challenge phase exposure for four days/week (duration not stated) for two weeks. The challenge phase was performed on a naïve site. Sensitization reactions were recorded for 23/30 people in the 10% group and 3/21 in the 1% group.

**CASRN 102-77-2 was considered a human sensitizer in this test.**

(3) Twenty human volunteers (gender and age distribution not reported) participated in a repeated-insult patch test with CASRN 102-77-2 (99% pure, 10% preparation in petrolatum). The robust summary stated that the 1953 Shelanski-Shelanski method was used. As described in the summary, the method was as stated in (2) above. However, the results were different – only 1/20 volunteers had a sensitization reaction. This may have been due to impurities since this test was performed with a CASRN 102-77-2 that was re-crystallized to get rid of impurities.

**CASRN 102-77-2 was not considered a human sensitizer in this test.**

(4) In a study to determine whether impurities in CASRN 102-77-2 may be responsible for the positive sensitization reactions observed in studies (1) and (2) above, human lymphocytes were obtained from seven volunteers that showed a positive sensitization reaction to the chemical. The lymphocytes were tested in vitro in a transformation assay with CASRN 102-77-2 (96% pure) and four other chemicals (2-mercaptobenzothiazole, morpholine, and two different oxidized impurities of CASRN 102-77-2). Although no details were provided on results, the summary concludes that the oxidized impurities were responsible for the sensitization response.

### ***Carcinogenicity***

Sprague-Dawley rats (50 animals/sex/dose) were exposed to CASRN 102-77-2 via the diet at the following doses 0, 5, 50, or 400 mg/kg-bw/day for 2-years. No mortalities were attributed to treatment. Animals in the 50 and 400 mg/kg-bw/day dose groups exhibited reduced food consumption and body weight gain compared to control animals. Increases in kidney and liver weights were also reported for mid- and high-dose animals. Microscopic examinations of any suspect neoplasms were conducted on all sacrificed animals and any animal that died during the study. Although no data were specifically provided in the summary, the submitter stated that there were no differences between test and control rats as to the organ system involved, type or classification of neoplasms, and that "... (T)he spectrum of neoplasms observed compared favorably to the historical data at this laboratory for rats of this age and strain".

**CASRN 102-77-2 was not carcinogenic in this assay.**

**Conclusions:** The acute toxicity of CASRN 102-77-2 is low in both rats (oral and inhalation routes of exposure) and rabbits (dermal route of exposure). In a two-year oral repeated-dose study with rats (dietary exposure), there were no adverse effects up to the highest dose of CASRN 102-77-2 tested. The NOAEL for systemic toxicity is 400 mg/kg/day (highest dose tested). A 21-day dermal study in rabbits showed changes in hematology measures at a dose of 2000 mg/kg/day CASRN 102-77-2; the NOAEL for systemic toxicity is 500 mg/kg/day. In a 28-day inhalation study with rats, there were no adverse effects observed up to the highest tested concentration of CASRN 102-77-2; the NOAEC for systemic toxicity is 0.0102 mg/L (highest concentration tested). There is no specific reproductive toxicity study available for CASRN

102-77-2; however, this endpoint has been satisfied for the purposes of the HPV Challenge Program because there is a developmental toxicity study (no developmental effects), the reproductive organs were evaluated in the two-year repeated-dose study (no effects observed), and there was a dominant lethal test in male rats which showed no effects up to the highest dose tested (500 mg/kg/day via gavage for 56 days). In the prenatal developmental toxicity study with CASRN 102-77-2 (in rats, via gavage), there was maternal toxicity observed at 300 mg/kg/day (decreased body weight gains and clinical signs); the NOAEL for maternal toxicity is 100 mg/kg/day. There was no developmental toxicity observed up to the highest tested dose; the NOAEL for developmental toxicity is 1000 mg/kg/day. CASRN 102-77-2 was negative for gene mutations when tested in bacteria *in vitro* but positive (with metabolic activation) when tested in mammalian cells *in vitro*. CASRN 102-77-2 did not induce chromosomal aberrations in mammalian cells *in vitro*. CASRN 102-77-2 was positive in a DNA repair assay in bacteria and equivocal in sister chromatid exchange studies in mammalian cells *in vitro*. CASRN 102-77-2 is irritating to the skin and eye in rabbits and is a skin sensitizer in humans. CASRN 102-77-2 was not carcinogenic in a two-year rat study.

<b>Table 3. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Human Health Data</b>	
<b>Endpoints</b>	<b>SPONSORED CHEMICAL Benzothiazole, 2-(4-morpholinylthio)- (102-77-2)</b>
<b>Acute Oral Toxicity LD<sub>50</sub> (mg/kg)</b>	<b>&gt; 7940</b>
<b>Acute Inhalation Toxicity LC<sub>50</sub> (mg/L)</b>	<b>&gt;0.09</b>
<b>Acute Dermal Toxicity LD<sub>50</sub> (mg/kg)</b>	<b>&gt;5010</b>
<b>Repeated-Dose Toxicity NOAEL/LOAEL Oral (mg/kg/day)</b>	<b>NOAEL = 400 (highest dose tested)</b>
<b>Repeated-Dose Toxicity NOAEC/LOAEC Inhalation (mg/L/day)</b>	<b>NOAEC = 0.0102 (highest concentration tested)</b>
<b>Repeated-Dose Toxicity NOAEL/LOAEL Dermal (mg/kg/day)</b>	<b>NOAEL = 500 LOAEL = 2000</b>
<b>Reproductive Toxicity NOAEL/LOAEL</b>	There were no effects on the reproductive organs in a two year study and a dominant lethal study was negative.

<b>Table 3. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Human Health Data</b>	
<b>Endpoints</b>	<b>SPONSORED CHEMICAL Benzothiazole, 2-(4-morpholinylthio)- (102-77-2)</b>
<b>Developmental Toxicity NOAEL/LOAEL Oral (mg/kg/day) Maternal Toxicity</b>	<b>NOAEL = 100 LOAEL = 300</b>
<b>Developmental Toxicity</b>	<b>NOAEL = 1000 (highest dose tested)</b>
<b>Genetic Toxicity – Gene Mutations <i>in vitro</i></b>	<b>Negative (bacteria) Positive (mammalian cells; with activation only)</b>
<b>Genetic Toxicity – Chromosomal Aberrations <i>in vitro</i></b>	<b>Negative (in mammalian cells)</b>
<b>Genetic Toxicity - Other</b>	<b>Positive (DNA repair test in bacteria) Equivocal (SCEs in mammalian cells)</b>
<b>Additional Information Irritation Sensitization Carcinogenicity</b>	<b>Slight (rabbit: eye and skin) Positive (human) Negative</b>

Measured data in bold text

#### 4. Hazard to the Environment

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 4.

##### *Acute Toxicity to Fish*

##### ***Benzothiazole, 2-(4-morpholinylthio)- (CASRN 102-77-2)***

(1) Rainbow trout (*Oncorhynchus mykiss*) were exposed to CASRN 102-77-2 under flow-through, open-system conditions for 144-hours at 0, 0.12, 0.25, 0.5, 1.0 and 2.0 mg/L plus a solvent control. Test was terminated following a 6-day exposure as no deaths had occurred for two consecutive days. DO, pH, temperature and chemical concentrations were measured routinely, measured chemical concentrations were lower than nominal concentrations and continued to decrease with study duration. Measured concentrations were not provided.

**96 LC<sub>50</sub> = 0.31 mg/L**

(2) Fathead minnow (*Pimephales promelas*) were exposed to nominal test concentrations of 0, 1.0, 1.8, 3.2, 5.6 and 10.0 mg/L plus a solvent control under static, closed-system conditions for

96-hours. DO, pH and water hardness were measured routinely. Temperature was maintained at 22 °C.

**96-h LC<sub>50</sub> = 3.5 mg/L**

***Acute Toxicity to Aquatic Invertebrates***

***Benzothiazole, 2-(4-morpholinylthio)- (CASRN 102-77-2)***

*Daphnia magna* were exposed to CASRN 102-77-2 at nominal concentration of 1.0-10.0 mg/L under static conditions for 48-hours. DO, pH, alkalinity and water hardness were measured and temperatures were maintained at 19 °C. Details of daphnid response were not provided.

**48-h EC<sub>50</sub> = 4.0 mg/L**

***Toxicity to Aquatic Plants***

***Benzothiazole, 2-(4-morpholinylthio)- (CASRN 102-77-2)***

Algae (species not specified) were exposed to in a closed system to CASRN 102-77-2 at nominal concentrations 0, 0.6, 1.0, 3.0, 6.0 or 10 mg/L for 72-hours. Maximum toxicity to algae occurred within 28-hours following exposure.

**72-h EC<sub>50</sub> = 2.0 mg/L (biomass)**

**72-h EC<sub>50</sub> = 2.0 mg/L (growth rate)**

**Conclusion:**

The acute toxicity data for fish exposed to CASRN 102-77-2 ranges from 0.31 to 3.5 mg/L. The acute toxicity data for aquatic invertebrates exposed to CASRN 102-77-2 is 4.0 mg/L. The acute toxicity to aquatic plants from exposure to CASRN 102-77-2 is 2.0 mg/L (biomass/growth).

<b>Table 4. Summary of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Aquatic Toxicity Data</b>	
<b>Endpoints</b>	<b>SPONSORED CHEMICAL Benzothiazole, 2-(4-morpholinylthio)- (102-77-2)</b>
<b>Fish</b>	
<b>96-h LC<sub>50</sub> (mg/L)</b>	<b>0.31 – 3.5 (n)</b>
<b>Aquatic Invertebrates</b>	
<b>48-h EC<sub>50</sub> (mg/L)</b>	<b>4.0 (n)</b>
<b>Aquatic Plants</b>	
<b>72-h EC<sub>50</sub> (mg/L)</b>	
<b>(biomass)</b>	<b>2.0 (n)</b>
<b>(growth rate)</b>	<b>2.0 (n)</b>

**bold = measured data (i.e., derived from testing); (n) = nominal.**

**5.        References**

Hinderer, RK, B. Myhr, DR Jagannath, SM Galloway, SW Mann, JC Riddle and DJ Brusick. 1983. Mutagenic evaluations of four rubber accelerators in a battery of in vitro mutagenic assays. *Environmental Mutagenesis*. Volume 5, pp. 193-215.