# 2',2'''-Dithiobisbenzanilide

135-57-9

OVERVIEW

2',2'''-Dithiobisbenzanilide came to the attention of the NCI Division of Cancer Biology following a review of chemicals that do not meet the criteria for inclusion in the United States (U.S.) Environmental Protection Agency (EPA) HPV Challenge Program even though their production volume in 1998 exceeded 1 million pounds. According to industry information, annual production or importation of 2',2'''-dithiobisbenzanilide in the European Union was 10 - 1,000 metric tons and this chemical was used between 1990 and 1994.

2',2'''-Dithiobisbenzanilide is used as a peptizing agent for natural and synthetic rubber and is the preferred peptizing agent when formulated with an activator and a clay diluent. These formulations are highly effective in shortening mastication times, reducing viscosity, and lowering costs during rubber processing. Occupational exposure to chemicals in the rubber industry is known to occur through inhalation and dermal contact. Another potential source of exposure is from the production and use of some polymers used to package food.

The current literature on 2',2'''-dithiobisbenzanilide is inadequate to characterize the toxicological effects of this chemical. Acute studies reporting irritation and sensitization data exist, but no subchronic, chronic, or genotoxicity tests were found in the available literature. This compound is very toxic to aquatic organisms and its release from industrial waste streams may be hazardous to the environment.

Potential breakdown products of 2',2'''-dithiobisbenzanilide are benzanilides and N-phenyl benzamides. These compounds have demonstrated various biological activities making the identification of metabolic products of 2',2'''-dithiobisbenzanilide a necessary component of assessing the toxicity of 2',2'''-dithiobisbenzanilide.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

In comments provided on January 25, 2006, Dr. John Walker supplied the following post-meeting information on Interagency Testing Committee (ITC) activities regarding 2',2'''-



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dithiobisbenzanilide. This chemical was added to Appendix B in the ITC's 56th Report (70 FR 61520, October 24, 2005) as one of 235 substances that were high production chemicals in the 1998 and 2002 Inventory Update Rules (IURs), but not in the 1990 or 1994 IURs. The ITC discussed a data-availability study of these 235 chemicals in its 56th Report and posted the results on its web site, http://www.epa.gov/opptintr/itc. 2',2'''-Dithiobisbenzanilide is also in the American Chemistry Council (ACC), Soap and Detergent Association (SDA), and Synthetic Organic Chemical Manufacturers Association (SOCMA) Extended HPV (EHPV) Program. The goal of the EHPV Program is to collect and publish health and environmental information on chemicals that did not qualify as HPV chemicals under the EPA's HPV Challenge program but have since reached the 1 million pound per year threshold. As a result of these activities, there are ongoing efforts to obtain and make available health effects and environmental data for this compound.

## DATA GAPS IDENTIFIED BY NCI

NCI has identified the following gaps in data needed to characterize 2',2'''-dithiobisbenzanilide.

- Complete toxicological characterization, including histopathology, for all major organs, including the heart. Based on the results of this subchronic study, the need for a 2-year carcinogenesis bioassay should be determined.
- Studies to identify metabolites and environmental degradation products, with characterization of their toxicities to appropriate species, as needed.
- Evaluation of the mutagenicity of 2',2'''-dithiobisbenzanilide using a standard battery of genotoxicity tests.
- Determination of the levels of 2',2'''-dithiobisbenzanilide in the environment, its degradation pathways, and any hazards associated with its release into the environment.

## NOMINATION OF 2',2'''-DITHIOBISBENZANILIDE TO THE NTP

Based on a review of the literature available as of December 15, 2005, and the recommendations of the Chemical Selection Working Group (CSWG) on that date, NCI nominates this chemical for testing by the National Toxicology Program (NTP) and forwards the following information:

- The attached Summary of Data for Chemical Selection



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- Copies of references cited in the Summary of Data for Chemical Selection and Dr. Walker's post-meeting comments
- CSWG recommendations to:

(1) Conduct studies to identify metabolites and environmental degradation products, with characterization of their toxicities to appropriate species, as needed.

(2) Evaluate the mutagenicity of 2',2'''-dithiobisbenzanilide and its degradation products using a standard battery of genotoxicity tests.

The CSWG assigned the priority for testing this chemical as high.

Because of the activities of the ITC and the data gaps involving possible ecological effects, this information is also being forwarded to the ITC.  Should future actions on this chemical be taken under the HPV Challenge Program, NCI requests that this information be considered as part of the public comments for this chemical.



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# SUMMARY OF DATA FOR CHEMICAL SELECTION

## CHEMICAL IDENTIFICATION

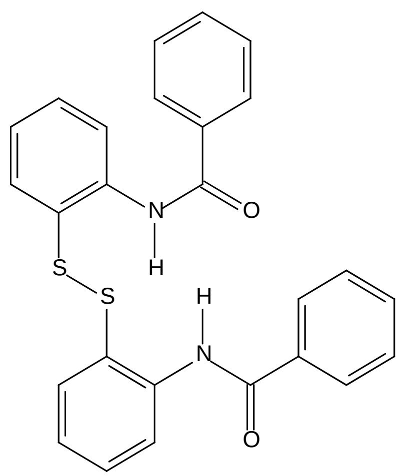
CAS Registry Numbers: 135-57-9

Chemical Abstracts Service Name: Benzamide, N,N'-(dithiodi-2,1-phenylene)bis- (9CI)

Synonyms and Trade Names: 2',2'''-Dithiobisbenzanilide; o-(benzoylamino)phenyl disulfide; bis(2-benzamidophenyl) disulfide; 2,2'-dibenzamidodiphenyl disulfide; o,o'-dibenzamido-diphenyl disulfide; 2,2'-dibenzoylaminodiphenyl disulfide; di-o-benzamidophenyl disulphide; EINECS 205-201-9; Noctizer SS; Peptazin BAFD; Peptisant 10; Pepton 22; Renacit 10 (ChemIDplus, 2004; Infochems Inc., 2005)

Structural Class: Benzanilide derivative

Structure, Molecular Formula, and Molecular Weight:




$C_{26}H_{20}N_2O_2S_2$

Chemical and Physical Properties:

Description: Pale yellow powder with mild odor (Thomas Swan MSDS, 2003)

Melting point: 136-144 °C (Thomas Swan MSDS, 2003)

Solubility: < 1% in water @ 20 °C (Thomas Swan MSDS, 2003)



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Density/Specific Gravity: ~ 1.35 @ 20 °C (Thomas Swan MSDS, 2003)

Reactivity: Flammable; capable of producing dust cloud explosions and emitting toxic fumes of carbon monoxide, carbon dioxide, nitrogen oxides, and sulfur oxides; incompatible with strong oxidizing agents (Thomas Swan MSDS, 2003)

O/W Partition Coefficient: 4.0 (Thomas Swan MSDS, 2003)

Technical Products and Impurities: 2',2'''-Dithiobisbenzanilide is not available from Sigma Aldrich or Fisher Scientific. 2',2'''-Dithiobisbenzanilide is sold as the pure compound and in several formulations, each with its own trade name. A list of these technical formulations is displayed in Table 1.

**Table 1. Some 2',2'''-Dithiobisbenzanilide Products for the Consumer Market**

| Product Name | Company | Description |
|---|---|---|
| Pepton 22 | Thomas Swan & Co Ltd (UK)<br>Swan Chemical Inc. (USA) | 2',2'''-Dithiobisbenzanilide ~ 100% purity (powder form) |
| Pepton 44 | Thomas Swan & Co Ltd (UK)<br>Swan Chemical Inc. (USA) | 2',2'''-Dithiobisbenzanilide with synergistic activator and inert dispersant<br>(powder form) |
| Pepton 44 Prills | Thomas Swan & Co Ltd (UK)<br>Swan Chemical Inc. (USA) | 2',2'''-Dithiobisbenzanilide with synergistic activator, inert carrier, and organic dispersants (prill form) |
| Pepton 44T Prills | Thomas Swan & Co Ltd (UK)<br>Swan Chemical Inc. (USA) | 2',2'''-Dithiobisbenzanilide with synergistic activator, inert carrier, and organic dispersants (dustless prill form) |
| Pepton 66 | Thomas Swan & Co Ltd (UK)<br>Swan Chemical Inc. (USA) | 2',2'''-Dithiobisbenzanilide with synergistic activator, inert carrier, and organic dispersants (pellet form) |
| Renacit 10 | Bayer AG | 2',2'''-Dithiobisbenzanilide (powder form) |
| Renacit 11 | Bayer AG | Activated 2',2'''-dithiobisbenzanilide absorbed on clay (powder form) |
| Renacit 11/WG | Bayer AG | 2',2'''-Dithiobisbenzanilide with activating additive and binder (granule form) |
| Akrochem Peptizer 66 | Akrochem Chemical Co. | Oil coated activated 2',2'''-dithiobisbenzanilide absorbed on clay (powder form) |



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| Product Name | Company | Description |
|---|---|---|
| Noctizer SS | OuchiShinko Chemical Industrial Co., Ltd | 2',2'''-Dithiobisbenzanilide (powder form) |

Source: Ash & Ash, 2004; Infochems Inc, 2005; Thomas Swan MSDS, 2003; Thomas Swan & Co. Ltd, 2005a,b,c,d

## EXPOSURE INFORMATION

Production and Producers:

*Manufacturing Process*. No information on the manufacturing process for 2',2'''-dithiobisbenzanilide was found in the available literature.

*Producers and Importers.* Chemical Sources International (2005) lists 4 U.S. suppliers of 2',2'''-dithiobisbenzanilide. ChemACX lists 2 suppliers selling 2 products; online vendors include Lancaster and TCI (ChemACX, 2005).

According to recent issues of chemical directories, 2',2'''-dithiobisbenzanilide is manufactured or distributed by Alfa Chem; J.T. Baker; Kenrich Petrochemicals Inc.; Pylam Products Co. Inc.; Rainbow Chemicals Co.; and Strem Chemicals Inc. (Chemcyclopedia, 2005).

Production/Import Levels:

2',2'''-Dithiobisbenzanilide is listed in the EPA Toxic Substances Control Act (TSCA) Inventory (ChemIDplus, 2005).

The EPA's Inventory Update Rule reports nonconfidential production ranges of chemicals every four years. The production/import levels of 2',2'''-dithiobisbenzanilide during the years 1986-2002 are listed in Table 2.

**Table 2. Production Levels of 2',2'''-Dithiobisbenzanilide**

| Year | Production Range (lbs.) |
|---|---|
| 1986 | 10,000 - 500,000 |
| 1990 | 10,000 - 500,000 |
| 1994 | 10,000 - 500,000 |
| 1998 | > 1,000,000 - 10,000,000 |
| 2002 | > 1,000,000 - 10,000,000 |

Source: EPA (2005a)



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2',2'''-Dithiobisbenzanilide is listed as an LPV chemical in the European Union, meaning that annual production was 10 - 1,000 metric tons at some time between 1990 and 1994. European producers are ABCR GmbH & Co KG, CHEMOS GmbH, and Thomas Swan & Co., Ltd. (ChemBuyersGuide.com, 2005; DWCP, 2004; European Chemicals Bureau, 2005).

For the 18-month period from July 31, 2003 to December 14, 2004, the Port Import/Export Reporting Service (PIERS) database reported eight imports of Pepton 22 with a cargo weight of 183,376 pounds. On July 31, 2003, the PIERS database reported an import of Pepton 44 prills with a cargo weight of 17,896 pounds (Dialog Information Services, 2005).

Use Pattern:

2',2'''-Dithiobisbenzanilide and pentachlorothiophenol are industrially important masticating agents. These chemicals reduce the viscosity of natural or synthetic rubber in a time- and energy-saving manner. Reducing viscosity promotes easier handling when molding rubber into its various end products. 2',2'''-Dithiobisbenzanilide is sold in many formulations under the trade names Pepton, Renacit, Akrochem Peptizer 66, and Noctizer SS. The various formulations are tailored to specific needs for both open and internal mixer mastication. Some formulations combine the masticating agent with inert carriers, e.g., clay diluent, and organic dispersants; such formulations are termed Prills. Prills that are designed to be non-dusting carry significant advantages in terms of industrial hygiene and non-dusting masticating agents based on 2',2'''-dithiobisbenzanilide are receiving increasing demand by the rubber processing industry for this reason (Ash & Ash, 2003; Buding & Hartmuth, 1997; Infochems Inc, 2005; Lautenberg, 1991; Ohm, 1997).

The majority of Pepton products, approximately 80-90%, are consumed by the tire industry. Smaller markets that utilize these products are rubber gloves for household use, latex gloves in the medical profession, printing screen rollers, and athletic shoes (Lautenberg, 1991).

2',2'''-Dithiobisbenzanilide has been used in the rubber industry for many years. Reports from the 1970s indicate that this chemical was used in the manufacture of tires, tubes, remolds, and retreads and in the manufacture of other solid rubber goods (rubber cables, belting and hoses, food processing equipment, aircraft de-icing equipment, automobile parts, sports goods and toys, and surgical and medical equipment). 2',2'''-dithiobisbenz-anilide was introduced as a peptizer in the initial stages of raw materials handling, weighing, and mixing operations that historically have been among the dirtiest in the industry (IARC, 1982).

Rubber materials that are produced using 2',2'''-dithiobisbenzanilide as a plasticizer have been approved by the FDA for repeated use in manufacturing, processing, transporting, or holding food. The amount of plasticizer used is not to exceed 30 percent by weight of the rubber product (FDA, 2004).

2',2'''-Dithiobisbenzanilide is cited in 6 U.S. patents and 2,2′-dibenzamidodiphenyl disulfide and Pepton 22 are both cited in 3 U.S. patents from 1976 to the present. Some of these patents suggest that this compound may be used in pharmaceutical formulations (United States Patent and Trademark Office, 2005).

<u>Human Exposure</u>:

*Occupational Exposure*. The Bureau of the Census estimates that in 1997, 247,800 people were employed by the rubber products industry, of which the tire industry employed 64,400. Approximately 57 percent of rubber products establishments, not including tire manufacturers, have fewer than 20 employees. Approximately 26 percent have between 20 and 100 employees, and only 3 percent have more than 500 employees. According to the 1997 Census of Manufacturers, there are 160 tire manufacturing plants in the U.S. During a 2002 survey, EPA identified 112 major tire manufacturing facilities along with 19 retreading operations. The two largest producers of tires, with 2,500 or more employees, accounted for approximately 54 percent of the tire production in 2001. In contrast, 46 percent of the facilities had less than 20 employees (EPA, 2005b).

Rubber product manufacturing is diverse, but there are several common processes: mixing, milling, extruding, calendering, building, vulcanizing, and finishing. The manufacturing process begins with the production of a rubber mix from raw or synthetic rubber, carbon black, oils, and chemicals intended to serve as processing aids, vulcanizing agents, activators, accelerators, age resistors, fillers, softeners, and specialty materials. The process of rubber mixing includes mixing, milling, antitack coating, and cooling. The appropriate ingredients are weighed in a compounding area and loaded into a Banbury mixer which combines the ingredients into a homogeneous mass of rubber. This mechanical action adds considerable heat to the rubber, so that it is then cooled and discharged to a mill to be formed into long sheets (EPA, 2005b).

Production of rubber products involves subjecting mixtures of hundreds of chemicals to heat, pressure, and catalytic action. As a result, the work environment may be contaminated with dusts, gases, vapors, fumes, and chemical byproducts. Workers may be exposed to these hazardous substances through inhalation and skin absorption (NIOSH, 1993).

In the rubber products industry, historically, raw materials handling, weighing, and mixing have been operations that have resulted in a substantial amount of human exposure. In some facilities the chemicals still sit in big open bins, increasing the potential for significant fugitive dust emissions. Most mixing facilities have eliminated this problem by purchasing their chemicals in small, preweighed, sealed bags put directly into the Banbury mixer. If this process is not used, fugitive emissions are also produced as the chemicals are loaded into the mixer. During the mixing phase when the raw polymer, either natural or synthetic, is mixed with chemical additives, exposure to these compounds can also occur from damage to containers or spillage during bulk transport, leaks in conveyor or piping systems used to transfer bulk materials, and inadequate maintenance of the ventilation system during mixing (Buding & Hartmuth, 1997; EPA, 2005b; IARC, 1982). Thus, the greatest release of 2',2'''-dithiobisbenzanilide into the occupational environment would occur in the compounding area and during the mixing process.

The National Occupational Exposure Survey (NOES), which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 12,863 workers, including 2,081 female workers, were potentially exposed to

2',2'''-dithiobisbenzanilide in the workplace (RTECS, 1997). The NOES database does not contain information on the frequency, level, or duration of exposure to workers of any chemical listed therein. This estimate does not reflect the anticipated increase in numbers of workers resulting from the current rise in demand, production, and use of 2',2'''-dithiobisbenzanilide.

Estimates from the NOES suggest that individuals working as machine operators in the following industries have more potential exposure to 2',2'''-dithiobisbenzanilide than other occupations: paper and allied products; printing and publishing; rubber and miscellaneous plastics products; and electric and electronic equipment (NOES, 2005).

*Consumer Exposure.* The rubber industry uses a special unit for expressing the components of rubber mixtures. They are presented in relation to 100 mass parts total rubber (parts per hundred rubber [phr]) or in wt-%. Masticating agents can contain a content of 90 to 10 wt% of 2',2'''-dithiobisbenzanilide. The use level of mastication agents/peptizers products in natural rubber products and synthetic rubber products is 0.1-0.5 phr and 1.0-3.0 phr, respectively. Based on 1996 estimates, the amount of 2',2'''-dithiobisbenzanilide used to manufacture a typical passenger car tire is <0.1 wt%. However, an analysis of rubber additives determined that approximately 99.5% of the mastication agent remains in the final rubber product (Ash & Ash, 2004; Buding & Hartmuth, 1997; California Integrated Waste Management Board, 2004; Umweltbundesant, 2003).

Based on the above information, the general population may be exposed to small amounts of unreacted 2',2'''-dithiobisbenzanilide leached from rubber products used to transport or package food and from rubber and plastic products designed for sale to the consumer market.

*Environmental Exposure.* Human exposure to 2',2'''-dithiobisbenzanilide may occur from its release into the environment during the manufacture, use, and disposal of products made from 2',2'''-dithiobisbenzanilide. Since small amounts of 2',2'''-dithiobisbenzanilide may be present in tires and other rubber goods, additional environmental exposure may occur in areas surrounding scrap tire yards or landfills.

Environmental Occurrence:

Additives used in the rubber industry may enter the environment through water, air, and soil. Water is the major pathway and the entry occurs whenever water comes into direct contact with the rubber product (cooling, heating, vulcanization, and cleaning operations). A survey of the wastewater discharges in the German rubber industry has divided these waste streams into sanitary, rainwater, rubber processing, metalworking, and others. It is estimated that 37 percent of the wastewater generated daily by the rubber industry is attributed to rubber processing (Umweltbundesant, 2003).

Small concentrations of Pepton 22 have been discharged into sewers from industrial wastewater in the United Kingdom (Barnabas, 2005). Pepton 22 is reportedly not readily biodegradable based on the carbon dioxide evolution test (Thomas Swan MSDS, 2003).

Acute toxicity values for 2',2'''-dithiobisbenzanilide in aquatic species are summarized in Table 3.

**Table 3. Ecotoxicity Values for 2',2'''-Dithiobisbenzanilide**

| Organism | Study Time | Toxicity Value |
|---|---|---|
| Rainbow trout | 96 hr | $LC_{50}$ >10 mg/l |
| Water flea (*Daphnia magna*) | 48 hr | $EC_{50}$ = 0.095 mg/l |
| Bacteria (*P. putida*) | -- | $EC_{10}/EC_{50}$ >8 mg/l |
| Algal inhibition (*S. Subspicaties*) | -- | $EC_{50}$ >8 mg/l |

Source: Thomas Swan MSDS, 2003

Regulatory Status:

No standards or guidelines have been set by NIOSH or the Occupational Safety and Health Administration (OSHA) for occupational exposure to or workplace allowable levels of 2',2'''-dithiobisbenzanilide. 2',2'''-Dithiobisbenzanilide was not on the American Conference of Governmental Industrial Hygienists (ACGIH) list of compounds for which recommendations for a Threshold Limit Value (TLV) or Biological Exposure Index (BEI) are made. The manufacturer, in the absence of a statutory limit set by a regulatory body, has recommended an 8-hour time-weighted average (TWA) long-term exposure limit (LTEL) of 2.5 $mg/m^3$ (Thomas Swan MSDS, 2003).

2',2'''-Dithiobisbenzanilide is not regulated under SARA Section 302 (Extremely Hazardous Substances), SARA Section 313 (Toxic Chemical Release Inventory), or CERCLA Section 103 (Hazardous Substances). 2',2'''-Dithiobisbenzanilide is classified as an environmentally hazardous substance and an aquatic toxicant under European regulations (Thomas Swan MSDS, 2003).



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TOXICOLOGICAL INFORMATION

Human Data:

Historically, cancer has been the chronic disease most frequently reported in cohort studies of rubber product workers. The risks for cancer and other chronic diseases in rubber products workers resulting from current exposure conditions are unknown. Toxicity data are also lacking for many chemical formulations found in tire and nontire manufacturing. The hazards that exist today may be different from those in the past because of changes in chemical formulations and the introduction of automated processes. In the absence of current epidemiologic and occupational exposure data, information about chemical formulations, and specific injury analysis, it is impossible to assess the risk posed to rubber products workers (NIOSH, 1993).

Contact dermatitis has been reported frequently among rubber workers and even more frequently among users of rubber products. Several NIOSH health hazard evaluations have reported contact dermatitis in tire and nontire plants, but most of the evaluations could not identify a specific chemical as the causative agent. Lack of information about sources of worker exposure including direct contact with bulk chemicals, processed stocks, and machinery contaminated with chemicals has contributed to the difficulty in determining the association between contact dermatitis and specific chemicals (NIOSH, 1993).

No epidemiological studies or case reports investigating the association of exposure to 2',2'''-dithiobisbenzanilide and cancer risks in humans were identified in the available literature.

2',2'''-Dithiobisbenzanilide has been described as a possible skin and respiratory irritant and may produce skin sensitization (Thomas Swan MSDS, 2003).

Animal Data:

No 2-year carcinogenicity studies of 2',2'''-dithiobisbenzanilide in animals were identified in the available literature.



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*Acute Toxicity*. The oral $LD_{50}$ for 2',2'''-dithiobisbenzanilide in rats is >4 g/kg and the dermal $LD_{50}$ for 2',2'''-dithiobisbenzanilide in rabbits is 10 g/kg. A mild irritating effect was observed in the eyes of rabbits 24 hr after being administered 500 mg of 2',2'''-dithiobisbenzanilide (RTECS, 1997; Thomas Swan MSDS, 2003).

Miles Inc. has made an EPA Section 8(e) submission to TSCA using data from their parent company Bayer AG regarding dermal sensitization studies on 2',2'''-dithiobisbenzanilide.

- The first study utilized the Buehler epicutaneous method on male guinea pigs where 25% concentrations of the test material in propylene glycol were used for the 1st, 2nd, and 3rd induction and for the challenge application. No dermal responses were reported in treated or control animals.
- Using the Magnusson and Kligman Maximization test, concentrations of 2',2'''-dithiobisbenzanilide used were as follows: intradermal induction - 2.5%; topical induction - 50%; first challenge - 50%; second challenge - 10%. After the first challenge, 12/20 treated animals exhibited positive reactions compared to 1/10 control animals. After the second challenge, 11/20 treated animals responded versus 1/10 control animals. It was concluded that 2',2'''-dithiobisbenzanilide is a medium-strong sensitizer when applied as a pure substance (Miles Inc, 1993).

*Prechronic/Subchronic Studies*. No subchronic studies of 2',2'''-dithiobisbenzanilide were found in the available literature.

*Chronic/Carcinogenicity Studies*. No 2-year carcinogenicity studies of 2',2'''-dithiobisbenz-anilide in animals were identified in the available literature.

Short-term Tests:

No *in vitro* or *in vivo* studies evaluating 2',2'''-dithiobisbenzanilide for mutagenic effects were found in the available literature.

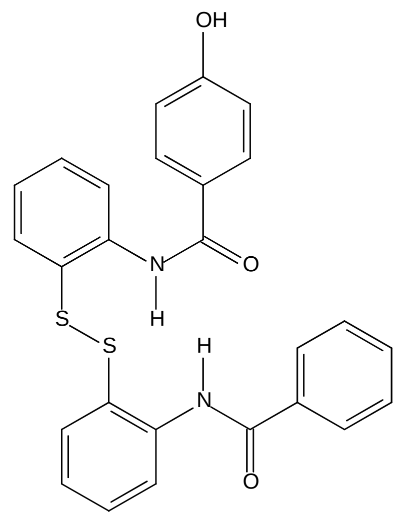
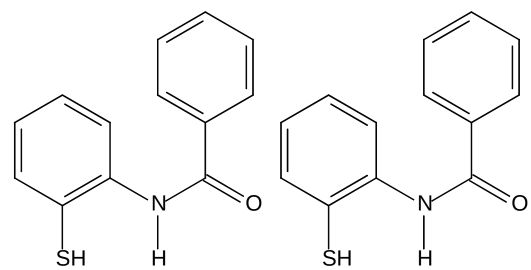
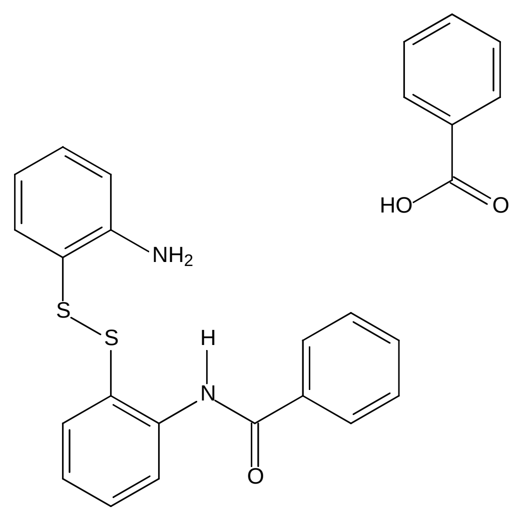
Metabolism:

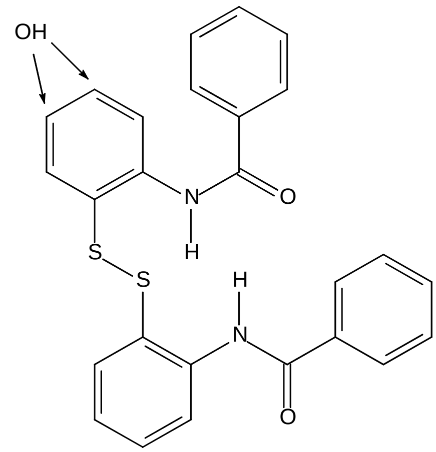
Although no studies on the metabolism of 2',2'''-dithiobisbenzanilide were identified in the available literature, the Chemical Selection Planning Group (CSPG) anticipates that 2',2'''-dithiobisbenzanilide may be converted to benzanilide derivatives that possess biological activity. These substances have been reported to produce antibacterial, antituberculous, antifungal, antiviral, antiprotozoan, anthelmintic, insecticidal, herbicidal, antitumor, immunosuppressive, hypnotic, anticonvulsive, anti-inflammatory, local anesthetic, anti-arrhythmic, vasodilating, anti-ulcerative, anti-androgenic, and hypoglycemic effects, but only a few of them have been introduced into practice (Kubicova & Waisser, 1997).

N-Phenyl benzamides are another group of compounds that may be produced following the metabolism of 2',2'''-dithiobisbenzanilide. These compounds have generated concerns because they may possess endocrine disruptor activity. In an estrogen receptor (ER) relative binding affinity assay, N-phenyl benzamides demonstrated low binding affinity for the ER receptor (Stauffer *et al*., 2000).

The metabolism prediction program, METEOR (LHASA Ltd., 2004), was used to determine potential breakdown products of 2',2'''-dithiobisbenzanilide. The proposed metabolites of 2',2'''-dithiobisbenzanilide are displayed in Table 4.

**Table 4. Metabolites Predicted for 2',2'''-Dithiobisbenzanilide by METEOR**

| Metabolic Reaction | Metabolites Formed |
|---|---|
| *para*-Hydroxylation of monosubstituted benzene compounds |   |
| Reductive S-S bond cleavage |   |
| Hydrolysis of acyclic carboxylic amides |   |

| | |
|---|---|
| 4-Hydroxylation of 1,2-disubstituted benzenes |   |

Other Biological Effects:

No additional relevant toxicological information was found for 2',2'''-dithiobisbenzanilide.

Structure/Activity Relationships:

Structurally related chemicals selected for 2',2'''-dithiobisbenzanilide were based on compounds that may be potential metabolites of the parent compound. Cleavage at the disulfide bond may produce 2-(benzoylamino)benzoic acid (CAS No. 579-93-1), 2'-methylbenzanilide (CAS No. 584-70-3), and benzanilide (CAS No. 93-98-1). Benzanilide was investigated for carcinogenicity in a limited 34-week study in female rats. Another potential cleavage site during the metabolism of 2',2'''-dithiobisbenzanilide is the N-phenyl bond. Breakdown products from this reaction may include diphenyl disulfide (CAS No. 882-33-7), a temporary enzyme-sulfhydryl blocking agent, and benzamide (CAS No. 55-21-0), an inhibitor of poly(ADP-ribose) synthesis. Both of these compounds have undergone mutagenicity and antimutagenicity testing. Toxicological information for these compounds is presented in Table 5.



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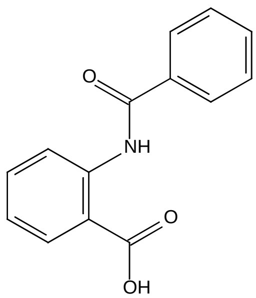
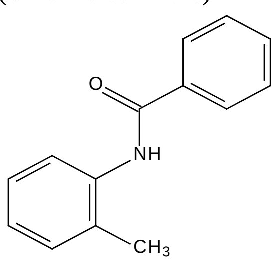
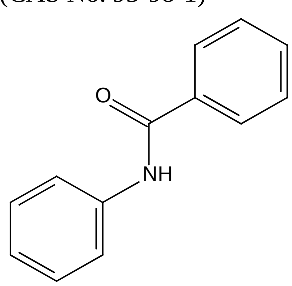
**Table 5. Toxicity Information on Chemicals Structurally Related to 2',2'''-Dithiobisbenzanilide**

| Compound/CAS No. | Mutagenicity/Carcinogenicity | Antimutagenicity |
|---|---|---|
| 2-(Benzoylamino)benzoic acid (CAS No. 579-93-1)  | No data found in available literature | No data found in available literature |
| 2'-Methylbenzanilide (CAS No 584-70-3)  | No data found in available literature | No data found in available literature |
| Benzanilide (CAS No. 93-98-1)  | Negative for carcinogenicity in a limited 34-wk study where 10 female rats (strain unspecified) were injected i.p. 3x/wk for 4 wks with 45 mg/kg bw benzanilide dispersed in 0.9% NaCl containing 1.75% gum acacia (Gutmann *et al.*, 1967) | No data found in available literature |



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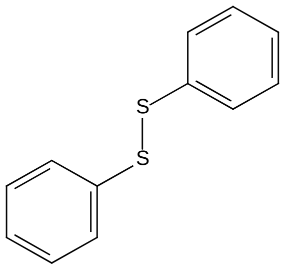
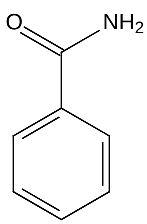
| Compound/CAS No. | Mutagenicity/Carcinogenicity | Antimutagenicity |
|---|---|---|
| Diphenyl disulfide<br>(CAS No. 882-33-7)<br> | Negative in *S. typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 with or without S-9 (Wild *et al*., 1983) | Inhibited $AFB_1$- and MMS-induced chromosome aberrations in rat bone marrow cells *in vivo* (Ito *et al*., 1997)<br><br>Suppressed micronuclei formation induced by MMC in peripheral blood reticulocytes of ICR mice *in vivo* and UV-induced mutation in *E. coli* B/r WP2 (Nakamura *et al*., 1997) |
| Benzamide<br>(CAS No. 55-21-0)<br> | Negative in *S. typhimurium* TA97, TA98, TA100, and TA1535 with or without S-9 (CCRIS, 2005)<br><br>Weakly positive in *E. coli* B/r WP2 (Rossman *et al*., 1991)<br><br>Induced MN in polychromatic erythrocytes of C57B1/6 female mice *in vivo* (Chieli *et al*., 1987)<br><br>Increased SCEs in human blood peripheral lymphocytes and in CHO cells *in vitro* (Das, 1985; Morris & Heflich, 1984; Oikawa *et al*., 1980; Park *et al*., 1983)<br><br>Weakly positive in producing mutations at the HGPRT locus in CHO cells (Heflich *et al*., 1985)<br><br>Produced spindle abnormalities, chromatin bridges, and micronuclei in mongoose kidney cells *in vitro* (Aravinda Babu *et al*., 1980) | Decreased the transformation frequency of mouse C3H 10T½ cells irradiated with UV light or exposed to MMS *in vitro* (Borek *et al*., 1984)<br><br>Decreased the transformation frequency of mouse C3H 10T½ cells and Syrian hamster embryo cells irradiated with X-rays or exposed to MNNG *in vitro* (Borek *et al*., 1984)<br><br>Decreased the mutation frequency and SCEs in Chinese hamster V79 cells exposed to MNNG *in vitro* (Bhattacharyya & Bhattacharjee, 1983; Roy *et al*., 1991) |

$AFB_1$ = aflatoxin $B_1$; MMC = mitomycin C; MMS = methyl methanesulfonate; MNNG = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine

Certain derivatives of diphenyl disulfide are known to cause hemolytic anemia in rats (Munday *et al*., 1990) and prolonged inhalation exposure to carbon disulfide can adversely affect the human nervous system and cardiovascular system (OSHA, 2005). Whether 2',2'''-dithiobisbenzanilide or any of its possible metabolites have similar effects is unknown.

Two SAR-based computer software programs were used as tools to assess the toxicity of 2',2'''-dithiobisbenzanilide. One program, named TOPKAT, uses robust, cross-validated models based on experimental data to calculate a probability value from 0.0-1.0 that a chemical will be positive for a certain endpoint. This program also incorporates a validity diagnostic that indicates if the predicted toxicity values may be accepted with confidence. Another SAR-based model, DEREK, uses structure alerts to predict the toxicity of a compound. The toxicity predictions made for 2',2'''-dithiobisbenzanilide by TOPKAT and DEREK are shown in Table 6.

**Table 6. Toxicity Predictions for 2',2'''-Dithiobisbenzanilide Using SAR-based Programs**

| **Toxicity Endpoint** | **Toxicity Prediction** |
|---|---|
| TOPKAT | |
| Carcinogenicity (male rat, NTP model) | 0.013 – Unlikely |
| Carcinogenicity (female rat, NTP model) | Prediction outside of confidence level |
| Carcinogenicity (male mouse, NTP model) | 0.942 – Probable |
| Carcinogenicity (female mouse, NTP model) | 0.076 – Unlikely |
| Carcinogenicity (male rat, FDA model) | 0.174 – Unlikely |
| Carcinogenicity (female rat, FDA model) | Prediction outside of confidence level |
| Carcinogenicity (male mouse, FDA model) | 0.000 – Unlikely |
| Carcinogenicity (female mouse, FDA model) | 0.950 – Probable |
| Weight of Evidence Carcinogenicity Call | Prediction outside of confidence level |
| Mutagenicity in the Ames assay | 0.000 – Unlikely |
| Developmental Toxicity | Prediction outside of confidence level |
| Skin Irritation | 1.000 – Probable |
| Skin Sensitization | Prediction outside of confidence level |
| DEREK | |
| Carcinogenicity | Plausible for mammalian carcinogenicity |
| Skin Sensitization | Plausible as mammalian skin sensitizer |

Source: Accelrys, Inc., 2004; LHASA Ltd., 2004

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