

Toxicological information

Toxicological studies about chlorine dioxide

Toxicological review

The toxicology of chlorine dioxide and chlorite following oral and inhalation exposure has been extensively reviewed. The most current reviews are the U.S. Environmental Protection Agency's *Toxicological Review of Chlorine Dioxide and Chlorite*¹ as also summarised in their *IRIS study*², and in the World Health Organization report on *Chlorine Dioxide Gas*³. The toxicology of chlorine dioxide, chlorite, and chlorate are also reviewed in the United Nations *International Programme on Chemical Safety, Disinfectants and Disinfectant By-products*⁴. Those studies demonstrate that **low levels of exposure to chlorine dioxide and its by-products do not cause adverse health effects.**

Judging from other applications of this chemistry, chlorine dioxide has proven to have **no mutagenic activity** when used to treat poultry water⁵ and leaves no detectable residue of chlorine dioxide, chlorite, chlorate or chlorine dioxide by-products on poultry carcasses after treatment⁶. Based on a review of the scientific data, the FDA has **approved chlorine dioxide as a secondary direct food additive**⁷ for use in poultry process water. Under this classification, the FDA is affirming that the **use of chlorine dioxide is safe** under the approved conditions of use.

The U.S. Agency for Toxic Substances and Diseases Registry's Public Health Statement⁸ on chlorine dioxide **does not list any effects on the skin.**

¹ U.S. Environmental Protection Agency. 2000. *Toxicological Review of Chlorine Dioxide and Chlorite*. EPA/635/R-00/007.

² U.S. Environmental Protection Agency. 2002. *Integrated Risk Information System (IRIS)*, available online at <http://www.epa.gov/iris/subst/o496.htm#studoral>.

³ World Health Organization. 2002. *Chlorine Dioxide (Gas)*; Concise International Chemical Assessment Document 37.

⁴ World Health Organization. 2000. *United Nations International Programme on Chemical Safety, Disinfectants and Disinfectant By-products*. Environmental Health Criteria 216.

⁵ Tsai, L.S., Wilson, R., Randall, V.. 1997. *Mutagenicity of Poultry Chiller Water Treated with Either Chlorine Dioxide or Chlorine*. J Agric Food Chem 45, 2267-2272.

⁶ U.S. Food and Drug Administration. 1995. *Secondary Direct Food Additives Permitted in Food for Human Consumption*. Federal Register 60 (42).

⁷ 21 CFR 173.300.

⁸ Agency for Toxic Substances and Disease Registry (ATSDR). 2002. *Toxicological Profile for chlorine dioxide and chlorite*. (Draft for public comment). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

The British Health and Safety Executive issued a document analysing the risks related to working with chlorine dioxide⁹. No or few data were available to assess the toxicological effects of chlorine dioxide. However, the following conclusions are drawn after the revision of all available data:

It is concluded that diluted aqueous ClO₂ (up to 5%) can cause eye irritation and slight skin irritation. It is also anticipated that ClO₂ would **not be a skin sensitizer**. There are **no reports of occupational asthma** associated with ClO₂.

On repeated dose toxicity, the HSE concludes that given the reactive nature of chlorine dioxide, it seems likely that **any health effects would be restricted to local responses**.

Referring to toxicity for reproduction, the HSE state that well-conducted studies in rats have shown that oral exposure at parentally toxic levels does **not impair fertility or development**. The HSE concludes that this is consistent with the view that as ClO₂ is a reactive gas it would be **unlikely to reach the reproductive organs** in significant amounts.

Toxicokinetics of chlorine dioxide

Ingested chlorine dioxide rapidly dissociates in the stomach into chlorite and chloride, and to a lesser extent, chlorate (EPA 2000). Thus, following ingestion of chlorine dioxide, the stomach experiences exposure to a mixture of chlorine dioxide, chlorite, chloride, and chlorate. Only 8 percent of chlorine dioxide remains in the stomach after 5 minutes (EPA 2000). At least 30 to 35 percent of the mixture of ingested chlorine dioxide and its dissociation products is absorbed from the gastrointestinal tract (EPA 2000). It is not clear whether the parent compound chlorine dioxide is absorbed, or just the chlorite, chlorate, or chloride ion degradation products. Peak plasma levels of adsorbed products are reached 1 to 2 hours after exposure. Once absorbed, the primary compounds found in the plasma are chloride (80 percent) and chlorite (20 percent) (EPA 2000). Thus, **the predominate dissociation product of chlorine dioxide in the body is the chloride ion (EPA 2000) and a significant portion of this product probably enters the chloride pool of the body (WHO 2000)**. Once absorbed, the chlorine dioxide dissociation products are distributed throughout the body, with the highest concentrations found in the blood, stomach, and small intestines. Chloride ion is the ultimate metabolite of chlorine dioxide, with urinary excretions being 87 percent chloride and 11 percent chlorite (EPA 2000). The parent compound, chlorine dioxide, is not detected in the urine. Excretion of the chlorine dioxide dissociation products is slow, compatible with the hypothesis that the bulk of the elimination is as chloride ion (WHO 2000b).

⁹ HSE Health & Safety Executive. *Chlorine Dioxide. Risk Assessment Document*. EH72/14.

Oral Exposure

The state of knowledge regarding chlorine dioxide and chlorite toxicity is fairly comprehensive, consisting of both sub-chronic and chronic studies, along with reproductive and developmental studies, and toxicokinetic and mechanistic information (EPA 2000, WHO 2002). Available data suggest that chlorine dioxide and chlorite have similar targets of toxicity and potencies. Therefore, the toxicity information for chlorite is relevant to deriving safe levels of exposure to chlorine dioxide. The results of **repeated oral exposure studies in rats and primates show no evidence of systemic toxicity associated with chlorine dioxide** (WHO 2002). Animals exposed for several months to either chlorine dioxide or chlorite ion in drinking water at concentrations above 50 to 100 ppm experience a reduction in red blood cell glutathione and a decrease in thyroid hormone level (EPA 2000). At lower concentrations, no adverse effects were noted. **Chlorine dioxide and sodium chlorite did not cause birth defects in laboratory animals, even at exceptionally high exposure levels** (WHO 2002).

Several studies have shown hematologic effects resulting from chlorine dioxide exposure in animals (EPA 2000). However, the EPA concluded that no consistent relationship has been demonstrated. While some studies found hematologic alterations in mice, rats, and monkeys, multiple other studies did not. The mechanisms of action whereby chlorine dioxide and chlorite produce hematologic effects are not completely understood (EPA 2000). Chlorite is thought to be the intermediate species most likely responsible for the hematologic effects of chlorine dioxide exposure (EPA 2000). After reviewing the data on hematologic effects, the EPA (2000) concluded that **oral hematologic effects, if any, seen in animals occur at doses higher than those for adverse neurodevelopmental effects** (EPA 2000).

Human Oral Exposure

Several assessments of the short-term (up to 3 months) oral toxicity of chlorine dioxide and chlorite in humans have been performed^{10 11}. In general, human studies have found **no adverse effects in individuals consuming low concentrations of chlorine dioxide or chlorite**. Short-term exposures to humans in the range 0.04 to 0.34 mg/kg have resulted in no physiologically relevant effects (EPA 2000, WHO 2002).

¹⁰ Lubbers, J.R., Chauhan, S., Bianchine, J.R.. 1981. *Controlled Clinical Evaluations of Chlorine Dioxide, Chlorite and Chlorate in Man*. Fundam Appl Toxicol 1:334-338.

¹¹ Lubbers, J.R., Chauhan, S., Miller, J.K.. 1984. *The Effects of Chronic Administration of Chlorine Dioxide, Chlorite and Chlorate to Normal Healthy Adult Male volunteers*. J Environ Pathol Toxicol Oncol 5:229-238.

Reviews of human exposure to chlorine dioxide as a water disinfectant show no adverse health effects (EPA 2000). Three assessments of long-term exposure to drinking water supplies containing chlorine dioxide in the range 0.25 to 1.11 ppm have been performed^{12 13 14}. The assessments focused on hematologic effects, serum chemistry alterations, and developmental toxicity. These studies found **no adverse effects in adults or neonates** (EPA 2000, WHO 2002). Chlorine dioxide is currently approved in the United States as an antimicrobial agent in drinking water in an amount not to exceed 0.8 ppm residual chlorine dioxide.

Michael et al. (1981) measured hematologic and serum chemistry parameters in 198 individuals for 10 weeks after initiation of a chlorine dioxide water treatment program. Blood samples were collected at the same times from a control group of 118 individuals not exposed to chlorine dioxide-treated drinking water. Chlorine dioxide in the drinking water ranged from 0.25 to 1.11 ppm, and chlorite concentrations ranged from 3.19 to 6.96 ppm (daily mean chlorite concentration was 5.21 ppm). The mean intake was estimated to be 10.3 mg/day (0.15 mg/kg-day). No hematologic or serum chemistry alterations were evident.

Tuthill et al. (1982) retrospectively compared infant morbidity and mortality data for a community with average monthly levels of 0.32 ppm of sodium chlorite added to drinking water post-treatment (chlorine dioxide levels were not known). Long-term exposure to chlorine dioxide—treated water did not adversely affect fetal, neonatal, postneonatal, or infant mortality or premature births, nor did it affect birthweight, sex ratio, or birth condition (Tuthill et al. 1982).

Kanitz et al. (1996) obtained data on infant birthweight, body length, cranial circumference, and neonatal jaundice from a community with drinking water disinfected with chlorine dioxide, sodium hypochlorite, or both; the levels of chlorine dioxide in the drinking water were less than 0.3 mg/L. The study concluded that infants of women who consumed drinking water treated with chlorine compounds during pregnancy were at higher risk for neonatal jaundice and had smaller cranial circumference and body length. Because of limitations in the study, the EPA (2000) concluded the study was flawed.

Based on the studies cited above, the EPA (2000) and the World Health Organization (2002) have concluded that human studies have demonstrated **no adverse effects in**

¹² Michael, G.E., Miday, R.K., Bercz, J.P.. 1981. *Chlorine Dioxide Water Disinfection: A Prospective Epidemiology Study*. Arch Environ Health 36:20-27.

¹³ Tuthill, R.W., Giusti, R.A., Moore, G.S.. 1982. *Health Effects Among Newborns After Prenatal Exposure to ClO₂-Disinfected Drinking Water*. Environ Health Perspect 46:39-45.

¹⁴ Kanitz, S., Franco, Y., Patrone, V.. 1996. *Associations Between Drinking Water Disinfection and Somatic Parameters at Birth*. Environ Health Perspect 104:516-520.

adults and neonates upon chronic oral exposure to chlorine dioxide in the range 0.04 to 0.15 mg/kg-day.

Inhalation Exposure

Several human occupational studies have examined the toxicity of inhaled chlorine dioxide (EPA 2000). These studies demonstrate that the respiratory tract is a sensitive target organ of exposure. The most frequently reported symptoms are wheezing, wheezing accompanied by breathlessness, and work-related wheezing. **No significant alterations in pulmonary function have been found** to be associated with human inhalation exposures. There are **no reports of skin sensitization or occupational asthma associated with exposure to chlorine dioxide** (WHO 2002). Animal studies of acute and subchronic inhalation toxicity of chlorine dioxide in rats and rabbits show alveolar congestion and hemorrhage, bronchial inflammation, and peribronchiolar edema. A no-observed-adverse-effect level (NOAEL) for these effects has not been identified; the lowest-observed-adverse-effect level (LOAEL) is 1 ppm (2.8 mg/m³) in rats exposed to chlorine dioxide 5 hours/day, 5 days/week for 2 months (EPA 2000). The 8-hour time weighted average (TWA) permissible exposure limit (PEL) for chlorine dioxide is 0.3 mg/m³ of air, which is approximately 0.1 ppm¹⁵.

Reproductive Effects

Several studies have evaluated the possibility of reproductive effects resulting from chlorine dioxide or chlorite exposure in rats (EPA 2000). For example, Carlton et al. (1991)¹⁶ administered daily gavage doses of 0, 2.5, 5, or 10 mg/kg chlorine dioxide to male Long-Evans rats for 56 days prior to mating and throughout the 10-day mating period. **No significant alterations in mortality, clinical signs, fertility rates, sperm parameters, length of gestation, prenatal deaths, mean litter size, or mean pup weights were observed.** The study identified a NOAEL of 10 mg/kg-day for reproductive effects in rats receiving gavage doses of chlorine dioxide.

Mobley et al. (1990)¹⁷ exposed female Sprague-Dawley rats to 0 or 100 ppm chlorine dioxide in the drinking water for 10 days prior to mating with unexposed males and during the gestation and lactation periods (until postconception days 35 to 42). At birth, the litter weight of the chlorine dioxide-exposed group was significantly lower

¹⁵ 29 CFR 1910.1000 Table Z-1.

¹⁶ Carlton, B.D., Basaran, A.H., Mezza, L.E.. 1991. *Reproductive Effects in Long-Evans Rats Exposed to Chlorine Dioxide*. Environ Res 56:170-177.

¹⁷ Mobley, S.A., Taylor, D.H., Laurie, R.D.. 1981. *Chlorine Dioxide Depresses T₃ Uptake and Delays Development of Locomotor Activity in Young Rats*. In: Jolly, R.L., et al. eds. *Water Chlorination: Chemistry, Environmental Impact and Health Effects*, Vol 6 Chelsea, M.I.: Lewis Publications, pp.347-358.

than that of controls. Chlorine dioxide exposure significantly decreased exploratory activity on postconception days 36 to 39, but not on days 39 to 41. This study identified a LOAEL of 14 mg/kg-day for decreased litter weight and exploratory activity.

After reviewing the data on reproductive effects, the EPA (2000) concluded that **reproductive effects seen in animal studies occur at doses higher than those for adverse neurodevelopmental effects**. The NOAEL for reproductive effects in rats is in the range of 10 to 14 mg/kg-day, while the NOAEL for neurodevelopmental effects in rats is 3 mg/kg-day (EPA 2000).

Neurodevelopmental Effects

Neurodevelopmental toxicity appears to be the most sensitive toxicological endpoint following oral exposure to chlorine dioxide or chlorite. **In-utero exposure to chlorine dioxide or postnatal gavage administration of chlorine dioxide in rats results in altered brain development** (decreases in brain weight, protein content, and cell number) **and decreased locomotor or exploratory activity** (EPA 2000). The LOAEL for these effects was 14 mg/kg-day chlorine dioxide (EPA 2000). The NOAEL for these studies was 3 mg/kg-day (EPA 2000).

The oral reference dose developed by the EPA is primarily based on neurodevelopmental effects observed in a two-generation drinking water study in rats¹⁸. In this study, 30 male and 30 female rats received drinking water containing 0, 35, 70, 300 ppm sodium chloride for 10 weeks and were paired for mating (35 ppm sodium chloride in drinking water corresponds to a dose of 3 mg chlorite/kg-day) (EPA 2000). Males were exposed throughout mating and then sacrificed. Exposure of the females continued through mating, pregnancy, and lactation until necropsy following weaning of their litters. Twenty-five males and females from each of the first 25 litters to be weaned in a treatment group were chosen to produce the F1 generation. The F1 pups were continued on the same treatment protocol as their parents. At approximately 14 weeks of age, they were mated to produce the F2a generation. Due to a reduced number of litters in the 70 ppm F1-F2a generation, the F1 animals were re-mated following weaning of the F2a generation to produce the F2b generation.

Reductions occurred in water consumption, food consumption, and body weight gain in both sexes in all generations at various times throughout the experiment, primarily in the 70 and 300 ppm groups. The authors attributed these reductions to

¹⁸ Gill, M.W., Swanson, M.S., Murphy, S.R.. 2000. *Two-Generation Reproduction and Developmental Neurotoxicity Study with Sodium Chlorite in the Rat*. J Appl Toxicol 20:291-303.

lack of palatability of the drinking water solution.

The EPA (2000) summarizes the effects as follows: “Effects observed included statistically significant decreases in pup body weight, absolute brain weight, liver weight, and lowered startle amplitude at the 28.6 mg/kg-day dose. Statistically significant decreases in auditory startle amplitude (F1 and F2 generations) and absolute and relative liver weights (FO and F1) occurred at 6 mg/kg-day. Although different responses were found for auditory startle (as indicated by measures of amplitude, latency, and habituation), this is not unexpected given that these measures examine different aspects of nervous system function and thus can be differently affected. Transient alterations in neurofunctional (or neurochemical) measures, such as in the auditory startle response, can occur without neuropathological changes and are considered of neurotoxic concern. Some of the effects observed at 6 mg/kg-day and 28.6 mg/kg-day occurred in both sexes and in more than one generation. These effects are considered toxicologically significant, which is consistent with EPA guidelines for reproductive, developmental, and neurotoxicity risk assessment.”

The mode of action for induction of adverse neurodevelopmental effects in rats is not known. It is also not known whether the rat is an adequate model for toxicity of chlorine dioxide and chlorite in humans. However, this species is widely used to characterize reproductive and developmental effects in humans (EPA 2000).

Mutagenicity

Chlorine dioxide has **no mutagenic activity** as an antimicrobial agent added to poultry chiller water (Tsai et al. 1997). However, chlorine dioxide has tested positive in some mutagenicity tests, but in the vast majority, it is negative (EPA 2000). Chlorine dioxide is positive in the Ames *Salmonella* assay, but in only one strain out of six (European Agency for Evaluation of Medical Products 2002).

Cifone and Myhr (1986, as cited in WHO 2002) found 0 to 65 micrograms μg) chlorine dioxide/ml to be mutagenic in the mouse lymphoma forward mutation assay, with and without metabolic activation. However, most other studies are negative. Rundell and Myhr (1986, as cited in WHO 2002) found 0 to 6 μg aqueous chlorine dioxide/ml not to be mutagenic in BALB/3T3 cells. Chlorine dioxide is negative as a clastogenic agent in a Chinese hamster ovary cell system and does not produce chromosomal aberrations or micronuclei in bone marrow of CD-1 mice following gavage doses of up to 16 mg/kg of body weight for 5 days (EPA 2000).

The limited **studies of mutagenicity of chlorine dioxide in *in viva* mammalian systems are all negative**. Using groups of male and female mice, **no mutagenicity was found in three separate studies involving chromosome aberrations in bone**

marrow cells, chromosome aberrations and micronucleus formation in bone marrow cells, and sister chromatid exchange (WHO 2002).

Thus, while chlorine dioxide is mutagenic when directly applied to the Ames *Salmonella* assay (EPA 2000), it is not mutagenic in most other applications (WHO 2002).

To see if chlorine dioxide or its organic and inorganic reaction by-products produced in poultry chiller water are mutagenic, Tsai et al. (1997) treated poultry chiller water with 100 ppm chlorine dioxide and then extracted the mixture and tested it in the Ames *Salmonella* assay. They found **chlorine dioxide to have no mutagenic activity** when used to treat poultry chiller water (Tsai et al. 1997).

Support for the negative mutagenicity of chlorine dioxide in poultry chiller water is provided by a study of the application of chlorine dioxide to Atlantic salmon and red grouper. Kim et al. (1999)¹⁹ treated cubes of Atlantic salmon and red grouper with 20 or 200 ppm aqueous chlorine dioxide for 5 minutes. Extracts of the treated fish cubes and test solutions were checked for mutagenicity using the Ames *Salmonella* assay. **No mutagenic activity was detected** in the treated fish samples or test solutions treated with chlorine dioxide.

Proteins in flour and bread, following oxidation with chlorine dioxide, are not mutagenic²⁰. Chlorine dioxide gas is used to treat flour during the baking of bread to produce bigger loaves. The gas is completely reduced to chloride and hypochlorite by reacting with flour. To test for mutagenicity of organic by-products in the flour, 3 extracts from treated flour (a predominantly lipid fraction [cyclohexane: ethanol extract], an amyolytic digest of delipidated flour, and the remaining insoluble residue) were examined for genotoxicity using bacterial assays (the Ames *Salmonella* assay, the Pol assay for DNA damage in *E. coli*, and the mouse lymphoma L5178Y TK+/-assay). **The chlorine dioxide treated extracts were not found to be mutagenic** (British Department of Health 1996).

In conclusion, chlorine dioxide can be considered having no mutagenic activity.

Carcinogenic assessment

The EPA (2000) concluded that the human carcinogenicity of chlorine dioxide and chlorite cannot be determined because no satisfactory human or animal studies

¹⁹ Kim, J.M., Du, W.X., Otwell, W.S., Wei, E.I.. 1999. *Determination of Chlorate and Chlorite and Mutagenicity of Seafood Treated with Aqueous Chlorine Dioxide*. J Agric Food Chem 47(9):3586-91.

²⁰ British Department of Health. 1996. *Report of the Committee on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment*. Available online at: <http://www.archive.official-documents.co.uk/document/doh/toxicity/chap-2a.htm>

assessing the chronic carcinogenic potential of chlorine dioxide have been located. The oral and inhalation databases are inadequate to assess the carcinogenicity of chlorine dioxide or chloride in humans or animals (EPA 2000). The United Nations also concluded “No tests of the carcinogenic activity of chlorine dioxide in experimental animals were identified in the scientific literature” (WHO 2000, WHO 2002).

Toxicological studies using Tristel's chlorine dioxide chemistry

Primary ocular irritation evaluation in rabbits²¹

The test article, at a dosage of 0.1 ml, was instilled into the conjunctival sac of the left eye of each of six New Zealand White rabbits. The right eye of the rabbits served as a control. Pre-selection ocular examinations were conducted approximately 24 hours prior to instillation of the test article. Following instillation, ocular examinations were conducted at 1, 24, 48 and 72 hours.

No visual signs of ocular irritation were observed in any of the rabbits at the 1, 24, 48 and 72 hour observation periods. No ocular changes were noted from pre-test in the control eye.

Body weights were taken at the initiation of the study.

These results suggest that the test article, in the activated form, is **not a primary ocular irritant** when instilled into the eye of albino rabbits.

Acute dermal toxicity study in rabbits²²

The test article was activated and applied at 2,000 mg/kg body weight to the closely clipped skin of adult New Zealand White rabbits (5/sex) under an occlusive wrap for 24 hours. Upon removal of the occlusive wrap, application sites were gently wiped with a damp clean disposable towel to remove any residual test article.

The rabbits were observed closely after dosing and at least twice daily for 14 days for mortality and other toxic effects, then sacrificed and subjected to gross necropsy. Body weights were recorded at study initiation, weekly thereafter and at termination of the study.

All 10 Group BGU1 rabbits gained weight and **no sign of systemic toxicity or mortality** was observed during the evaluation. There were no gross necropsy findings attributable to treatment with the test article.

Since no deaths occurred, the **acute LD₅₀ value** from dermal exposure to activated test article under occlusion is **greater than 2,000 mg/kg** body weight in albino rabbits.

²¹ T.P.S. Inc., Virginia, USA, November 1997

²² T.P.S. Inc., Virginia, USA, November 1997

Primary dermal irritation evaluation in rabbits²³

One-half ml aliquots of the test article were applied to intact closely clipped skin on the back of each of six New Zealand White rabbits. The application sites were occluded for four hours, then unwrapped and gently cleaned.

Application sites were evaluated for erythema/eschar and oedema for all animals according to the method of Draize within 30-60 minutes after unwrapping and again at 24, 48, and 72 hours after unwrapping. Body weights were recorded at initiation and termination of the animal phase.

No visual indications of erythema or oedema were present on any of the six rabbits within 30-60 minutes after the occlusive wrap was removed. There were no visible signs of skin irritation at the 24, 48 and 72 hour observation periods following unwrapping.

All of the rabbits gained weight during the 72 hour observation period.

These results suggest that the test article, when applied in activated form, is **not a primary irritant to the skin** of albino rabbits.

48 hour human patch test for primary irritation²⁴

The objective of the study was to compare the primary skin irritation potential of Tristel (chlorine dioxide) with that of Nu-Cidex (a peracetic acid product).

In a pilot study, three subjects received occlusive patch applications of both test products on their upper arm for 4 hours. Tristel produced minimal irritation but Nu-Cidex produced severe irritation with erythema, oedema and vesiculation.

The pilot study subjects then received a further 23 hour patch application of Tristel which produced minimal irritation. In the main study, 25 subjects received two occluded patch applications of Tristel on the upper arm which produced minimal irritation.

Due to the severity of the reactions observed with Nu-Cidex it was not included in the main study.

²³ T.P.S. Inc., Virginia, USA, November 1997.

²⁴ Consumer Product Evaluation Centre, Ledbury, Herefordshire, March 1995.

Under the conditions of this test, Tristel showed **negligible irritation potential** whereas Nu-Cidex was a strong irritant.

Dermal sensitisation evaluation in the guinea pig²⁵

The activated test article was evaluated for dermal sensitisation potential in young adult female guinea pigs. Animals in Groups BGV₃ (activated test article) and BFU₅ (DNCB positive control) were dosed dermally with 0.4 ml applied to the same close-clipped (24 hours prior to dosing) site on the left shoulder area once a week for 3 weeks. Two weeks following the last induction dose, a single challenge dose was administered dermally to the close-clipped left rear side of each of the group BGV₃ and BFU₅ animals. Animals in Group BFU₂ (DNCB control) were dosed dermally once with 0.4 ml applied to the close-clipped left rear side.

All challenge application sites were read and scored at 24 and 48 hours following dosing. Comparisons were made of the scores of the challenge applications from guinea pigs which received the inductive applications to those which received only the challenge application.

Skin reactions to DNCB, a known dermal sensitiser, were also evaluated and used to confirm the validity of the test method.

Based on this method, these results suggest that activated test article is **not a sensitising agent** when administered by dermal application to the close-clipped skin of albino guinea pigs. DNCB was clearly identified as a dermal sensitiser by this method.

All guinea pigs gained weight during the study. **No mortality occurred and no signs indicative of systemic toxicity were observed.**

Acute oral toxicity evaluation with rats²⁶

Ten albino Sprague Dawley derived rats (5/sex) were dosed by oral gavage with activated test article at a dosage of 5,000 mg/kg body weight. Observations were made up to six hours after dosing and twice daily for 14 days for signs of toxicity, behavioural changes or mortality. All surviving rats were subjected to a complete gross necropsy following the 14 day observation period.

²⁵ T.P.S., Inc, Virginia, USA, June 1998.

²⁶ T.P.S., Inc, Virginia, USA, June 1998.

There were **no deaths or remarkable clinical signs** observed following dosing or at any time during the 14 day observation period.

No remarkable gross necropsy findings were noted in any of the rats at study termination. Nine of the 10 rats gained weight during the study.

The **LD₅₀** for the activated test article is **greater than 5,000 mg/kg** in the Sprague Dawley rat.

Toxicological classification and values

On the basis of this information, Tristel's chlorine dioxide chemistry can be classified as:

- Skin contact – low risk: no sensitisation, no known hazard
- Eye contact – low risk: no sensitisation, no known hazard
- Inhalation – low risk: no sensitisation, no known hazard
- Ingestion – low risk: no known hazards

The related toxicological indicator values are:

- Occupational Exposure short term (OES) = 0.3 ppm short term
- Occupational Exposure long term (OEL) = 0.1 ppm long term
- 8-hour time weighted average (TWA) permissible exposure limit (PEL) = 0.3 mg/m³ of air
- LD₅₀ (dermal exposure; rabbits) = >2,000 mg/kg
- LD₅₀ (oral toxicity; rats) = >5,000 mg/kg