CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
HUMAN HEALTH ASSESSMENT BRANCH

SUMMARY OF TOXICOLOGY DATA

Dinitrogen Tetroxide, Nitrogen Dioxide

Chemical Code # 6123, 6124**, Document Processing Number (DPN)**: 53219

SB 950 # NA
2/5/15

DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect indicated.
Chronic toxicity, dog:	No study submitted nor required at this time.*****
Oncogenicity, rat:	No study submitted nor required at this time.*
Oncogenicity, mouse:	No study submitted nor required at this time.*
Reproduction, rat:	No study submitted nor required at this time.*
Developmental toxicity, rat:	No data gap, possible adverse effect indicated.
Developmental toxicity, rabbit:	No study submitted nor required at this time.*
Gene mutation:	No data gap, possible adverse effect indicated.
Chromosome effects:	No data gap, no adverse effect indicated.
DNA damage:	No data gap, no adverse effect indicated.
Neurotoxicity:	No data gap, possible adverse effect indicated.

Toxicology one-liners are attached.
All record numbers for the above study types through 281093 (Document No. 53219-0009) were examined. This includes all relevant studies indexed by DPR as of 2/5/15.

In the 1-liners below:
indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.

File name: T150205
Revised by T. Moore, 2/5/15

* The active ingredient is to be used in a closed system thus greatly minimizing any exposure. It is being evaluated for use as a gaseous sterilant (antimicrobial) for non-critical medical devices, laboratory equipment, isolators, pharmaceuticals and pharmaceutical equipment. The data

base provided for review is drawn from the public literature as the active ingredient has been extensively evaluated as an airborne pollutant

NOTE: The following symbols may be used in the Table of Contents which follows:
* = data adequately address FIFRA requirement
† = study(ies) flagged as “possible adverse effect”
N/A = study type not currently required

This record contains summaries of studies. Individual worksheets may be useful for detailed assessment.

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METABOLISM AND PHARMACOKINETICS

Study not submitted nor required at this time.

GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

Acute oral toxicity, rat

Study not required.

Acute dermal toxicity

Study not required.

Acute inhalation toxicity, rat

Guideline study not submitted; appropriate acute inhalation hazard category has been determined from data in the public literature.

Primary eye irritation, rabbit

Study not required at this time.

Primary dermal irritation

Study not required at this time.

Dermal sensitization

Study not required at this time.

SUBCHRONIC STUDIES (units of mg/kg/day unless specified)

Oral toxicity, rat:

Study not required.

Oral toxicity, non-rodent:

Study not required.

Inhalation toxicity, rat:

Study not submitted nor required at this time.

Dermal toxicity, 21/28-day or 90-day, rat:

Study not required.

CHRONIC STUDIES

Chronic, rat

53219-0009; 281093; “Effect of Long-Term Nitrogen Dioxide Exposure on Rat Lung: Morphological Observations”; (Kubota, K., M. Murakami, S. Takenaka, K. Kawai, H. Kyono;

Basic Medical Sciences Division, National Institute for Environmental Studies, Yatabe, Tsukuba, Ibaraki, 305, Japan and National Institute of Industrial Health, Tama-ku, Kawasaki 214, Japan; *Environmental Health Perspectives* 73, 157-169 (1987)); Three cohorts of male JCL/Wistar rats/group were exposed whole body 24 hours/day to 0, 0.04 (0.08 mg/m^3 at STP), 0.40 (0.77 mg/m^3) or 4.0 ppm (7.66 mg/m^3) of nitrogen dioxide for 9, 18, or 27 months. As the primary site of exposure, the lungs of each animal were fixed *in situ,* excised and examined by both light and electron microscopy. Morphometric measurements were performed which determined the arithmetic mean thickness of the air-blood barrier (AMT) , the volume density of the alveolar wall components (VdAWT), the mean number of alveolar cells in a unit area and the volume density per cell. For the 4 ppm exposure group, hypertrophy and hyperplasia of the bronchial mucosa and the thickening of the walls through the bronchopulmonary junction of the alveolar duct were evident by 9 months of exposure. Cellular infiltration and an increase in the presence of Clara cells were noted as well. There was a steady progression in the severity of the lesions with interstitial fibrosis and hyperplasia of the epithelium evident at the bronchopulmonary junction up to 27 months of exposure. No emphysema was evident. In the morphometric evaluations, there was a decrease in the numbers of type I epithelial cells with a concomitant increase in cell volume. There was a comparative increase in the ratio of type II epithelial cells to type I cells after 9 months of exposure. By 18 months, the number and volume of type II cells and the interstitium had increased further. This was designated the Phase I process. In Phase II, between 18 and 27 months, the interstitial edema was replaced by the *de novo* synthesis of collagen fibers. For the 0.4 ppm exposure group, these lesions were present as well but their progression was slower. For the 0.04 ppm exposure group, a slight increase in the thickness of the air-blood barrier was apparent by 27 months. **Possible adverse effect:** Cellular alterations at the bronchopulmonary junction in the lungs. **Rat Chronic Toxicity NOEL**: <0.04 ppm (based upon the presence of thickening of the air-blood barrier in the alveolar walls of the lungs of the 0.04 ppm exposure group). **Summary Report** (Moore, 12/4/14)

53219-0009; 281093; "Studies on the Biochemical Effects of Nitrogen Dioxide, IV. Relation between the Change of Lipid Peroxidation and the Antioxidative Protective System in Rat Lungs upon Life Span Exposure to Low Levels of NO_2"; (Sagai, M., T. Ichinose, K. Kubota; Basic Medical Sciences Division, The National Institute for Environmental Studies, P.O. Tsukuba, Ibaraki, 305, Japan; *Toxicology and Applied Pharmacology* 73, 444-456 (1984); Three cohorts of male JCL/Wistar rats/group were exposed whole body 24 hours/day to 0, 0.04 (0.08 mg/m^3 at STP) , 0.40 (0.77 mg/m^3) or 4.0 (7.66 mg/m^3) ppm of nitrogen dioxide for 9, 18, or 27 months. There was no treatment-related effect upon the survival of the study animals. Lung homogenates were assayed for total protein, total lipid, nonprotein sulfhydryls and thiobarbituric acid (TBA) reactants. Glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione S-reductase, superoxide dismutase and disulfide reductase activities were measured. In addition, *in vivo* lipid peroxidation was determined by assaying for ethane and pentane in the exhaled air. Additional groups of animals were exposed to 0, 0.04, 0.12 or 0.4 ppm of the test material and ethane and pentane were assayed after 6, 9 and 18 months of exposure. Pentane evolution was used as a measure of microbial-mediated lipid oxidation. Exposure to NO_2 resulted in increased levels of TBA reactants in the lungs of the 4 ppm exposed animals after 9 months of exposure and in the lungs of the 0.4 and 4 ppm animals after 18 months of exposure. The concentration of nonprotein sulfhydryls increased in the lungs of the 0.4 and 4 ppm exposed animals after 9 and 18 months of exposure. The glutathione peroxidase (H_2O_2 substrate) activity was reduced by 11% for the 4 ppm animals after 9 and 18 months of exposure. Glucose 6-phosphate dehydrogenase activity was elevated in the lungs of the 4 ppm exposed animals at both 9 and 18 months. Gluthathione S-tranferases, aryl- and aralkyl-, activities were less than the control

values for the 0.4 and 4 ppm groups after 18 months of exposure. Significant ethane production was noted in a dose-dependent manner for all of the exposure groups after 9 and 18 months of exposure. Interestingly this effect was evident for the 0.04 and 0.4 ppm groups after 27 months of exposure but ethane production for the 4 ppm group had significantly declined by then. The production of ethane in an exposure-related manner was confirmed in the additional exposure groups. Pentane evolution was not appreciably enhanced. The report authors surmised that NO_2-induced lipid peroxidation increased as the activity of the anti-oxidative protective enzymes (glutathione S-tranfersases and glutathione peroxidase (H_2O_2 substrate)) declined. Ethane production increased in an exposure-related manner until significant thickening of the air:blood barrier and fibrosis had occurred in the lungs of the 4 ppm group. **Possible adverse effect:** deleterious structural changes in the lungs; **Rat Chronic Toxicity NOEL**: <0.04 ppm (based upon increased ethane production for the 0.04 ppm exposure group); **Summary report.** (Moore, 12/5/14)

Chronic, dog

Study not submitted nor required at this time.

Oncogenicity, rat

Study not submitted nor required at this time.

Oncogenicity, mouse

Study not submitted nor required at this time.

GENOTOXICITY

Gene mutation

53219-0009; 281093; "Induction of Mutations and Chromosomal Aberrations in Lung Cells following *In Vivo* Exposure of Rats to Nitrogen Oxides"; (K. Isomura, M. Chikahira, K. Teranishi, K. Hamada; Public Health Institute of Hyogo Prefecture, Hyogo ku, Kobe 652, Japan; *Mutation Research* 136, 119-125 (1984)); Five male Sprague-Dawley rats/group were exposed whole body to 0, 8 (15.3 mg/m^3 at STP), 15 (28.7 mg/m^3), 21 (40.2 (mg/m^3) or 27 ppm (51.7 mg/m^3) of NO_2 for 3 hours. The animals euthanized at 18 hours post-exposure and primary lung cell cultures were established. In the mutagenicity study, cell survival was assessed by culturing 4 plates/rat of 200 cells each for 7 days, fixing and counting the number of colonies per plate. For the mutation assessment, 8 plates/rat of 2x10^5 cells each were incubated for 5 hours and then exposed to the selective agent, ouabain (1 mM) for 14 days. At that time the colonies were fixed and stained with Giemsa. For the chromosomal aberration assessment, lung cell suspensions derived from the control and the 8 and 27 ppm exposure groups were plated in triplicate and incubated for 3 days. Colcemid (0.05 µg/ml) was added to the medium at 5 hours prior the end of the incubation. The cells were then treated with a hypotonic buffer, fixed with acetic acid:methanol (1:3) and stained with Giemsa. No positive control was included in the study. There was a significant increase in the mutation frequency noted for the 15 ppm exposure group and above. In the chromosomal aberration assessment, there was an increase in the incidence of aberrations for the 27 ppm exposure group. **Possible adverse effect:** increased mutagenicity and incidence of chromosomal aberrations. **Summary Report**. (Moore, 12/9/14)

Chromosome damage

53219-0009; 281093; "Observations on Mouse Chromosomes following Nitrogen Dioxide Inhalation"; (P.C. Gooch, H.E. Luippold, D.A. Creasia, J.G. Brewen; Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN; *Mutation Research* 48, 117-120 (1977)); Male C3H mice

were exposed whole body to 0.1 (0.19 mg/m^3 at STP), 1 (1.91 mg/m^3), 5 (9.6 mg/m^3), or 10 ppm (19.1 mg/m^3) for 6 hours. Blood samples were drawn by cardiac puncture from each animal immediately after the exposure and 1 and 2 weeks later. The blood was cultured for 52 hours with the addition of cholchicine after 48 hours of incubation. The animals were maintained for 8 weeks post-exposure. At that time, spermatocytes were recovered from each animal and subjected to chromosomal analysis. There was no increase in the incidence of chromosomal aberrations in the leucocytes or spermatocytes. No positive control was used in the study. **No adverse effect indicated. Summary report.** (Moore, 12/9/14)

DNA damage or miscellaneous effects

53219-0009; 281093; "Genotoxic Activity of 1, 3-Butadiene and Nitrogen Dioxide and Their Photochemical Reaction Products in Drosophilia and in the Mouse Bone Marrow Micronucleus Assay"; (K. Victorin, L. Busk, H. Cederberg, J. Magnusson; Institute of Environmental Medicine, Karolinska Institutet, S-104 01 Stockholm, Sweden, Swedish Food Administration, S-751 26 Uppsala, Sweden, and Department of Genetic and Cellular Toxicology, The Wallenberg Laboratory at the University of Stockholm, S-106 91, Stockholm, Sweden; *Mutation Research* 228, 203-209 (1990)); In the somatic mutation and recombination test with Drosophila, females with the recessive marker of multiple wavy hairs (mwh/mwh) were mated with males having the recessive lethal wing marker of flare (flr^3/ser). Serrate in this case is the dominant genetic marker. Seventy two-hour old larvae were exposed to 50 ppm (95.7 mg/m^3 at STP) of NO_2 for 5 or 19 hours. Seven day later, the adults were collected and preserved in 70% ethanol. The wing is comprised of cell clones which express one or another phenotype. The wings of these adults were scored as to the presence of a clone of one of the phenotypes. The number of spots (clones) per wing were scored. It was not apparent as to how this assay delineated a positive mutagenic response. No positive control was included in the assay. No mutagenic response was evident for the larvae exposed to NO_2. In the micronucleus study, male NMRI mice were exposed to 20 ppm (38.3 mg/m^3) of NO_2 for 23 hours. A positive control was exposed to 5% vinyl chloride under the same conditions. At 30 hours post-exposure initiation, the animals were euthanized and bone marrow was extracted from the femurs of each animal. The erythrocytes were recovered, mounted on slides, stained with a combination of May-Grunwald stain followed by Giemsa and dried. One thousand polychromatic erythrocytes (PCE) were scored per animal for the presence of micronuclei (MNPCE). The ratio of PCE per normocytic erythrocytes (NCE) was determined as well. The percentage of micronucleated PCEs was not increased as consequence of the exposure to the test material. The positive control was functional. **No adverse effect indicated**. **Summary report.** (Moore, 12/9/14)

REPRODUCTIVE TOXICITY, RAT

Study not submitted nor required at this time.

DEVELOPMENTAL TOXICITY

Rat

Study not submitted nor required at this time.

Rabbit

Study not submitted nor required at this time.

NEUROTOXICITY

Acute neurotoxicity, rat

Study not submitted nor required at this time.

90-day neurotoxicity, rat

Study not submitted nor required at this time.

Developmental neurotoxicity, rat

53219-0009; 281093; "Postnatal Effects of Maternal Exposure to Nitrogen Dioxide"; (S. Tabacova, B. Nikiforov, L. Balabaeva; Institute of Hygiene and Occupational Health, Medical Academy, Sofia, Bulgaria; *Neurobehavioral Toxicology and Teratology* 7, 785-789 (1985)); Twenty mated female Wistar rats were exposed whole body to 0, 0.05, 0.10, 1.0, or 10 mg/m^3 of NO_2 for 6 hours/day throughout gestation (until day 21). The viability and body weight of the offspring were monitored up to 2 months post natal. Post-natal neurodevelopment was assessed using numerous functional observational endpoints. Post-natal development of specific liver metabolizing enzymes was assessed. Other biochemical parameters possibly affected by NO_2 exposure were likewise monitored. The mean body weight and viability of the 1.0 and 10 mg/m^3 offspring were less than the control group values up through one month post-natal. Developmental landmarks of incisor eruption and eye opening were significantly delayed for the 1.0 and 10 mg/m^3 exposure groups. In the neurodevelopmental assessment, the percentage of pups which could successfully perform the surface and air righting test was reduced for the pups in 1.0 and 10 mg/m^3 groups up through post-natal day 18. In the negative geotaxis assessment on day 9 post-natal, the percentage of success was significantly reduced for the 0.1, 1.0 and 10 mg/m^3 pups. The 10 mg/m^3 pups demonstrated a significant deficient in both the auditory startle reflex and hindlimb support. In the open field assessments on day 9 post-natal, the 1.0 and 10.0 mg/m^3 pups demonstrated reduced motility and a prolonged period of passivity. These two groups and the 0.1 mg/m^3 pups exhibited postural and gait defects. By day 14, only the postural and gait defects were evident for these three groups. Upon reassessment on day 30, reduced motility and exploratory behavior were evident for the male pups in the 1.0 and 10 mg/m^3 groups and persisted through day 60. In the assessment of liver metabolism, the hexobarbital sleeping time was increased for the pups in the 1.0 and 10 mg/m^3 groups through day 21. For other biochemical parameters in the liver on day 30, lipid peroxides were elevated for the 1.0 and 10 mg/m^3 groups and cytochrome P450 content, aminopyrine-N-demethylase activity and oxygen consumption were reduced for the 10 mg/m^3 pups. Overall, the neurodevelopmental deficits were evident in the pups of the 0.1 mg/m^3 exposure group and above. **Possible adverse effect:** neurodevelopmental deficits of reduced motility and prolonged passivity. **Rat Developmental Neurotoxicity NOEL:** 0.05 mg/m^3 (based upon the incidence of neurodevelopmental deficits noted of the pups in the 0.1 mg/m^3 exposure group); **Summary report.** (Moore, 12/8/14)

Delayed neurotoxicity, hen

Study not submitted nor required at this time.

IMMUNOTOXICITY

Study not submitted nor required at this time.

ENDOCRINE DISRUPTOR STUDIES

Study not submitted nor required at this time.

SUPPLEMENTAL STUDIES

53219-0009; 281093; "Nitrogen Dioxide Exposure: Effects on Airway and Blood Cells"; (M.W. Frampton, *et. al.*; Departments of Medicine, Environmental Medicine and Biostatistics, University of Rochester School of Medicine, Rochester, NY, Division of Infectious Diseases,

University of Texas Medical Branch, Galveston, TX; *American Journal of Physiology. Lung Cellular and Molecular Physiology* 282, L155-L165 (2001)); Human subjects were exposed on separate occasions to air and to 0.6 (1.15 mg/m^3 at STP) and 1.5 ppm (2.87 mg/m^3) of NO_2 for 3 hours. Pulmonary function testing was performed prior to each exposure and immediately after the exposure's conclusion. At 3.5 hours post-exposure, blood was drawn from each subject and bronchial (BL) and alveolar (AL) lavages and brush biopsies were performed in a bronchoscopic procedure. The blood was assayed for hematocrit, hemoglobin and red blood cell indices and for total and differential white blood cell counts. Flow cytometry was employed to differentiate subsets of lymphocytes. The BL and AL samples were assessed in regard to total cell count and viability. Lymphocytes in the AL were phenotypically sorted by flow cytometry. Aliquots of BL, AL and lung epithelial cells were exposed to influenza or respiratory syncytial (RSV) viruses and assayed for viability in the presence of these viruses. Exposure to NO_2 did not affect any of the pulmonary function parameters. For the red blood cells, the only parameter which was affected was the hematocrit. The value was reduced by 4% after the high exposure in comparison to that of the control. There appeared to be an exposure-related reduction of specific white blood cells in the differential leucocyte count. However, statistical significance was not achieved. The CD4+/CD8+ ratio of lymphocytes was increased in an exposure-related manner for the males and decreased for the females. In the BL, polymorphonuclear neutrophils increased in an exposure related manner. In the AL, the percentage of CD4+ lymphocytes increased in an exposure-related manner. *In vitro* exposure of lung epithelial cells to the two viruses resulted in a reduced viability related to directly to exposure to NO_2. This effect was statistically significant for RSV. There was no exposure-related effect on albumin or total protein content in the BL or AL which indicated no effect on epithelial cell permeability as a consequence of the exposure. **No adverse effect indicated. Summary report.** (Moore, 12/10/14)