Applied Occupational and Environmental Hygiene

ISSN: 1047-322X (Print) 1521-0898 (Online) Journal homepage: https://www.tandfonline.com/loi/uaoh20

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To cite this article: William K. Alexander , G. Bruce Briggs , Kenneth R. Still , Warren W. Jederberg , K. MacMahon , W. H. Baker & C. MacKerer (2001) Toxicity of 2,6-Di-*tert*-butyl-4-Nitrophenol (DBNP), Applied Occupational and Environmental Hygiene, 16:4, 487-495, DOI: 10.1080/10473220117206

To link to this article: https://doi.org/10.1080/10473220117206

Published online: 30 Nov 2010.

Applied Occupational and Environmental Hygiene
Volume 16(4): 487–495, 2001
1047-322X/01 $12.00 + .00

Toxicity of 2,6-Di-*tert*-butyl-4-Nitrophenol (DBNP)

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U.S. Navy submarines reported a yellowing of metal surfaces on their internal surfaces. The yellowing was initially identified on the painted steel bulkheads but further examination indicated that it was not limited to steel surfaces and included bedding, thread tape, Formica, plastisol covered hand-wheels, and aluminum lockers. Crew members also reported to the medical department that their skin turned yellow when they came in contact with these contaminated surfaces and requested information on the effects of exposure. Studies conducted by General Dynamics' Electric Boat Division (EBD) determined that the agent was 2,6-Di-tert-butyl-4-Nitrophenol (DBNP).(1) 2,6-Di-butylphenol (DBP) is an antioxidant additive used in lubricating oils and hydraulic fluids. In the enclosed atmosphere of a submarine, the oil mist could be spread throughout the boat by venting the lube oil to the atmosphere. Submarines use electrostatic precipitators (ESP) to clean the air of particulate materials. During passage through the ESP, oil mist containing DBP is nitrated to DBNP, which is then moved throughout the boat in the ventilation system. Analysis of the EBD data indicated 24-hour exposure concentrations to be in the range of <3.0 to 122 ppb in the laboratory and submarine settings. Submarine crews may be exposed to these concentrations for as many as 24 hours/ day for 90 days during underway periods. Toxicity studies regarding the oral and dermal uptake of DBNP were conducted. From the literature the lethal dose to 50 percent of the population (LD_{50}) of DBNP (rat) was reported by Vesselinovitch et al. in 1961 to be 500 mg/kg.(2) Our studies indicated that the LD_{50} is in the range of 80 mg/kg in the rat. Our work also includes dermal absorption studies, which indicated that DBNP is not well absorbed through intact skin. Within this study, no no-observable adverse effect level (NOAEL) or lowest observable adverse effect level (LOAEL) was identified. Calculation of a reference dose was completed using standard methods based on the LD_{50} as a numerator with several uncertainty and modifying factors. EBD's determination of airborne concentrations aboard submarines fall in the range of these anticipated allowable concentrations and could indicate significant chronic exposures. No adverse effects from DBNP exposures have been reported to date.

Keywords DBNP, Submarine, DBNP Toxicity, DBP

In 1993, U.S. Navy submarine crews began to notice yellowing of the bulkheads and other structures during underway periods and sea trials. The yellowing was reported to General Dynamics Electric Boat Division (EBD) as a failure of the paint system. EBD conducted a thorough investigation and found that the yellowing was not caused by failure of the paint coating system, but, instead, by 2,6-Di-*tert*-butyl-4-Nitrophenol (DBNP). DBNP is formed when lubricating oil mist containing 2,6-Di-*tert*-butylphenol (DBP), an antioxidant additive in many synthetic lubricating oils and hydraulic fluids, passes through an electrostatic precipitator, and is nitrated. DBNP is an intensely yellow crystalline material. Submarine personnel coming into contact with DBNP on bedding, bulkheads, or other surfaces noted yellowing of their skin and requested information from the medical department regarding the hazards of exposure. EBD's data indicated that although the yellowing was most pronounced in the engineering compartments, DBNP moves throughout the submarine in the ventilation system. This finding indicates that DBNP could reasonably be expected to accumulate on dishes, glasses, flatware, bedding, and other items leading to ingestion as well as dermal contact and inhalation as potential routes of exposure. Analysis of the EBD data indicated identifiable DBNP concentrations in the air at several locations in the submarine. Concentrations ranged from <3.0 to 13 ppb. EBD also conducted laboratory simulations of the submarine environments finding DBNP concentrations as high as 122 ppb.

In 1961, Vesselinovitch et al. reported the LD_{50} for DBNP as 500 mg/kg in Sprague-Dawley rats.[2] Holder et al. concentrating on the ingestion route of exposure, reported that DBNP was toxic in mammals and that approximately 30 percent of the oral dose was absorbed.[3] As a pilot study to determine the range of the LD_{50} in rats, a new acute oral study was conducted by the Naval Health Research Center Toxicology Detachment (NHRC/TD), using Fischer 344 rats with corn oil as the vehicle. The intent of this study was to validate the findings, specifically to replicate the LD_{50}, of the Vesslinovitch study.[2] Findings of the new study indicated the LD_{50} in rats at 80 mg/kg making DBNP significantly more toxic then previously recognized.[4] Differences in the findings were attributed to differences in rat strain and the vehicle. In 1999, a second acute oral study, sponsored by NHRC/TD, was conducted in Sprague-Dawley rats to validate findings of the 1998 study and for further comparison to the 1961 study. Results of the second study supported the LD_{50} at 80–100 mg/kg in rats.[5]

While DBNP yellowing seemed to be confined to the closed environment of submarines during underway periods, anecdotal information was also received from petroleum manufacturers and suppliers that refineries were noting yellowing of the walls and equipment. Additionally, submarine services from other nations are now beginning to report yellowing aboard their submarines.

There is little information on DBNP in the published literature. DBNP is a yellow crystalline powder with a melting point at 157°C and a molecular weight of 251. It is soluble in organic solvents, but relatively insoluble in water. Reviews of the literature indicated that very little was known regarding the toxicity of DBNP. This chemical was proposed as a commercially available miticide in the late 1950s. A 1961 study by Vesselinovitch et al. determined the LD_{50} values for guinea pigs, mice, and rats in oral and intraperitonneal exposures.[2] In that study, they determined the oral LD_{50} in Sprague-Dawley rats to be in the 400–500 mg/kg range. DBNP was indicated as relatively non-toxic to humans. The 1961 study was conducted in Sprague-Dawley rats using carboxymethylcellulose as the vehicle. Rats were exposed to an oral bolus (0.2 ml/20 g of body weight) at each concentration of DBNP. No specific discussion referencing the manner of death was provided. Conclusions indicated that DBNP dosages were cumulative with increased mortality among animals given daily doses as small as 1/25 of the LD_{50} for an unspecified period of time, believed to be less than 14 days. No discussion of the potential for enterohepatic circulation was provided. Vesselinovitch et al. reported a gender difference in DBNP toxicity with the LD_{50} in females being slightly higher than in the males.[2]

In a 1970 study, Holder et al. reported on excretion of DBNP.[3] They concluded that Vesselinovitch et al. had shown that DBNP was highly toxic to mammals in the 1961 study.[2] They indicated a significant relationship between DBNP and 3,5-Di-*tert*-butyl-hydroxy toluene (BHT), another antioxidant additive in lubricating oils and hydraulic fluids. This study was conducted using a 20 mg oral dose of radio-labeled carbon fourteen (^{14}C) DBNP. They reported that DBNP was poorly absorbed from the gut with approximately 30 percent of the oral dose excreted unchanged. The absorbed DBNP was excreted as a glucuronide conjugate. No other metabolites were identified. They found that DBNP administered orally was excreted primarily through feces and urine with a small percentage in the bile. The findings were equivocal on enterohepatic circulation.

In a 1997 technical report, the Naval Medical Research Institute's Toxicology Detachment (NMRI/TD) characterized the metabolism, distribution, and toxicity of DBNP.[6] They reported that DBNP had a low toxicity in rats, but that the effects of exposure were cumulative. They indicated that DBNP is cleared from the system very slowly and that repeated exposures could further reduce excretion. They indicated that enterohepatic circulation was the likely explanation for the delayed elimination in that, during the time period for the excretion of DBNP and its metabolite, water consumption and urine output remained the same as controls. They also reported finding a glucuronide conjugate excreted in urine, feces, and bile. No other metabolites were identified. They reported that the toxicity of DBNP was linked to inhibitory effect on the synthesis of adenosine triphosphate (ATP). Histologic evaluation found fatty patches in the livers of animals exposed to higher doses (>100 mg/kg). They concluded that DBNP might be less toxic in humans than in other species.

DBP is an antioxidant additive used by many lubricating oil and hydraulic fluid manufacturers. Figure 1 is a diagrammatic representation of the DBP molecule. Electrostatic precipitators are used to clean the air of particles aboard submarines and in industry. During operation of the machinery, the lubricating oil is heated. Vents on the equipment allow for the expanding vapor to escape to the environment. DBP-containing oil mists pass through the electrostatic precipitators (ESP) and are nitrated to form DBNP. Figure 2 is a diagram of the DBNP molecule.

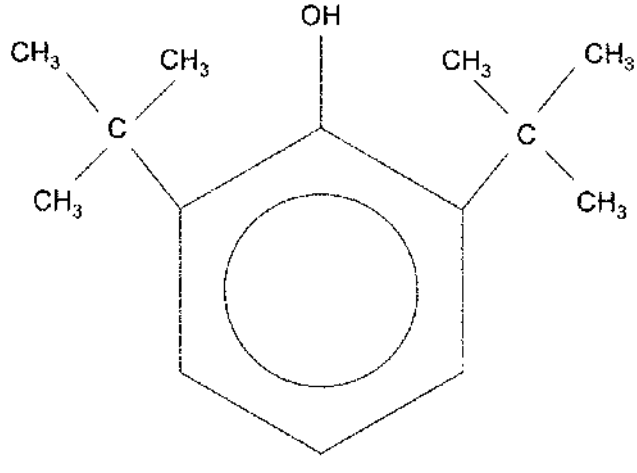

FIGURE 1
2,6 di-*tert*-butylphenol.

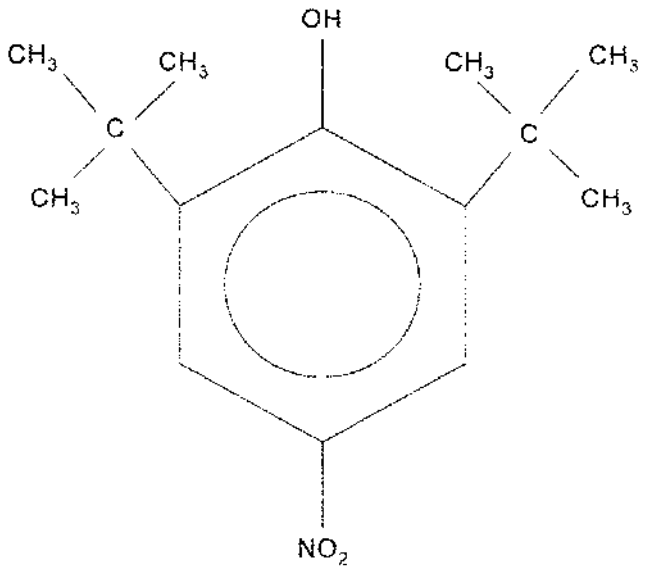

FIGURE 2
2,6 di-*tert*-butyl-4-nitrophenol.

EBD personnel reported strong and direct correlation between the concentration of DBNP and ESP production of oxides of nitrogen. EBD also reported a strong inverse correlation between DBNP production and the operating efficiency of the ESP which is directly related to the number of hours of operation since the last cleaning. Furthermore, they found a direct correlation between the concentration of DBP in the oil and DBNP in the air. Additionally, EBD identified 2,6-Di-*tert*-butyl-Methylphenol (DBMP) as an ancillary product in the oil and the air, but did not link it to the yellowing phenomenon.

Submarines have ESP equipment onboard, both in the machinery compartments and in the ventilation systems. EBD reasoned that additional DBNP could be generated when un-reacted DBP moved through the ventilation system's vent fog precipitators. Through this mechanism, EBD indicated that DBNP aerosol could be expected to move throughout the ventilation system to all compartments in the submarine, providing venues for inhalation, dermal and oral routes of exposure. DBNP settling on surfaces could also lead to dermal contact. DBNP settling on flatware, dishes, and glassware could lead to ingestion.

MATERIALS AND METHODS

Airborne Concentration

EBD personnel constructed an apparatus to simulate submarine environments in a laboratory setting. This simulator included a 50-gallon (U.S.) vented oil storage tank, connecting duct work, and an $8' \times 8' \times 6'$ enclosure with an electrostatic precipitator inside. Samples were collected from the lubricating oils and analyzed using gas chromatography/mass spectrometry (GC/MS), with a flame ionization detector (FID), to determine the concentration of DBP in the bulk material. Concentrations of DBP were <1.0 percent for all lubricating oils analyzed. A vent fog ESP and oil storage tank were set up to simulate the DBNP generation process. The lubricating oil, a 2190 TEP (synthetic turbine oil MILSPEC-L-17331), was heated to >145°F. As the oil expanded in the tank due to heating, oil mist escaped through the vent and passed through the ESP in the simulator. DBNP concentrations were determined at the outflow of the ESP and in the ambient air within the simulator. Experiments were also conducted to evaluate the potential for surface nitration of DBP in the laboratory simulator. Additional measurements were made in a number of locations aboard operational submarines during underway periods.

Air samples were collected using activated charcoal tubes and analyzed using GC/MS. Air and wipe samples were also collected aboard submarines using methods developed by EBD personnel. Paint scrapings collected from the yellowed area were also collected and analyzed, using GC/MS to confirm the presence of DBNP.

Toxicity Testing

Test animals used in these studies were Fischer 344 rats (male and female), New Zealand white rabbits (male), and Sprague-Dawley rats (male only). Test animals were procured through approved vendors and were quarantined for a period of 14 days after delivery. Animals were housed in high-efficiency particulate air (HEPA)-filtered laminar-flow tents, except during dosing and weighing. Rats were individually housed in clear plastic cages with Cel-Sorb Plus laboratory bedding. Rabbits were housed in wire bottom, stainless steel cages. Water and feed (Purina Formula #5008 [rats] and Purina Rabbit Chow #5320) were provided ad libitum, except the 12 hours immediately prior to dosing. Animal rooms were maintained at temperatures from 21–25°C and light/dark cycles were set at 12-hour intervals.

In the first acute oral and dermal toxicity study, male and female Fischer 344 rats and New Zealand white rabbits were procured and quarantined for 14 days prior to exposure. Using DBP and nitric acid, DBNP was synthesized by a method developed in the NMRI/TD laboratory.[6] The DBNP was analyzed by GC/MS and determined to be 99 percent pure with 1.0 percent un-nitrated DBP. Figure 3 is the chromatogram of the analysis. In the acute oral portion of this project, five male Fischer 344 rats were gavaged at each dose level with DBNP in corn oil at concentrations of 500, 275, 100, and 50 mg/kg of body weight. Five female Fischer 344 rats were gavaged at each dose level with DBNP in corn oil at concentrations of 450, 275, 100, or 50 mg/kg of body weight.

In the second acute oral study, male Sprague-Dawley rats were procured and quarantined for 14 days prior to exposure. DBNP purity was again determined by GC/MS and found to be 97 percent DBNP, with the remaining 3.0 percent unreacted DBP. Five male rats per dosage were gavaged with DBNP in corn oil at 250, 98, 78, and 62.5 mg/kg of body weight. To evaluate the effects of the change in vehicle, five male Sprague-Dawley rats were gavaged with DBNP in a 2.0 percent aqueous

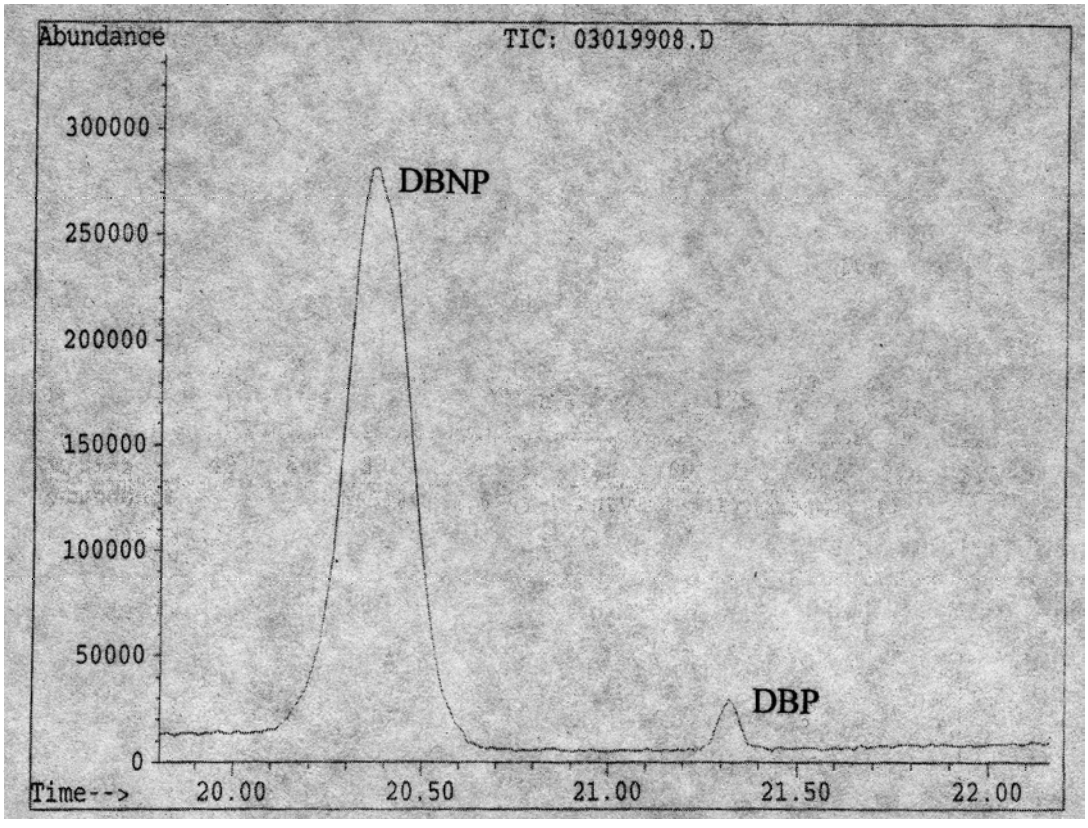

FIGURE 3
Gas chromatography/mass spectroscopy (GC/MS) analysis of DBNP.

carboxymethylcellulose solution with a DBNP concentration of 98 mg/kg of body weight. The Vesselinovitch et al. study cited a 0.2 percent aqueous carboxymethylcellulose vehicle, which was unachievable during this study.[2] The manufacturer of the carboxymethylcellulose recommended a 2.0 percent aqueous solution vehicle.

With regard to the dermal toxicity, five New Zealand white rabbits were selected to determine the dermal exposure and coefficient of transfer for DBNP. Rabbits were weighed and clipped. DBNP, 2.0 grams/kg of body weight, was applied directly to their backs and held in place by elastoplast tape. Rabbits were exposed for 24 hours. Animals were observed for 14 days, and weighed on days one, seven, and 14 post-treatment.

Statistical analysis was conducted using Microsoft Excel and Slidewrite software. Changes in body weight were compared using a two way repeated measures of variance. Changes in body temperature values were evaluated using a t-test or Mann Whitney Rank Sum Test. A probability 0.05 or less inferred significant change from controls. LD_{50} calculation were performed using the method of Weil (1952).

RESULTS

During a ten-day sampling period in the laboratory, EBD reported results of nine air samples of DBNP concentrations from the outflow of the ESP inside the simulator. Concentrations ranged from <2.0 to 122.0 ppb with a mean concentration of 36.0 ppb and a standard deviation (sd) of 43.3. Sixteen measurements of the DBNP concentrations in the ambient air within the simulator were made during the same ten day period. Results ranged from <2.0 to 37.0 ppb DBNP with a mean of 15.5 ppb (sd = 11.9). More than 150 samples were collected from three submarines during underway periods. Results indicated ambient airborne concentrations of DBNP ranging from <1.0 to 13.0 ppb with a mean of 2.9 ppb (sd = 1.2), with the highest reported concentrations found in the main engineering compartments. No data was provided regarding sampling at the outflows of submarine ESPs.

In the Fisher 344 rats using corn oil as the vehicle, mortality was 100 percent for male and female rates in the highest dose groups (500 mg/kg, and 450 mg/kg), respectively. Within 30 minutes of dosing all treated animals were prostrate in their cages with rapid respiration. Several animals convulsed immediately prior to death. At the time of death the animal's bodies were in a severe state of rigor. The body temperature of one rat taken immediately after death was 105°F (normal temperature is 96.4–99.7°F). Hearts were palpably firm. Histologic analysis of skeletal muscle demonstrated multifocal mild degeneration of the muscle fibers with occasional contraction bands. Similar findings were made in the cardiac musculature.

TABLE I
Comparison of DBNP dose, mortality, and time to death among male Fisher 344 rats

Dose (mg/kg)	Mortality (%)	Time to death (maximum)
500	100	2 hr, 38 min
275	100	3 hr, 12 min
100	80	4 hr, 16 min
50	0	—

Fisher 344 rats dosed at 275 mg/kg also suffered 100 percent mortality, dying within 3.25 hours or 3.8 hours for the males and females, respectively. Elevated body temperature, and rigor were noted. Histologic evaluations indicated similar changes in skeletal and cardiac musculature.

Animals dosed at 100 mg/kg DBNP suffered 80 percent and 60 percent mortality for the males and females, respectively. Prostration, rigor, elevated temperature, were all seen in these groups. Results of histologic examinations were similar to those of the higher dose groups.

Mortality among the animals dosed at 50 mg/kg DBNP was zero (0%). Prostration, rigor, and elevated temperatures were again seen. Histologic evaluations indicated less significant changes in muscle and other cell types.

Tables I and II indicate the mortality and time to death at each dose level for the male and female Fisher 344 rats, respectively.

There were no deaths among the control groups. Animals lost weight immediately after the gavage procedure, but the duration of weight loss was short. The animals returned to normal weight quickly and did not indicate any other signs of adverse reactions to the procedures.

Among the Sprague-Dawley (SD) rats, mortality was 100 percent for those dosed at 250 mg/kg DBNP in corn oil. Hyperthermia, prostration, lethargy, and rapid respiration were seen prior to death. Hepatic and pulmonary congestion was a common finding. Degenerative and necrotic changes were noted in individual hepatocytes and kidney ductal epithelial cells.

Mortality among animals dosed to 98 mg/kg was 60 percent with two animals dying within 4 four hours and one dying overnight. Hyperthermia, rigor, prostation, and rapid breathing were evident. Body temperatures rose to as much as 108°F between the time of dosing and death. The peak temperature for the two animals that survived was 104°F, and their temperatures returned to normal within 30 hours of dosing.

TABLE II
Comparison of DBNP dose, mortality, and time to death among female Fisher 344 rats

Dose (mg/kg)	Mortality (%)	Time to death (maximum)
450	100	3 hr, 48 min
275	100	3 hr, 48 min
100	60	4 hr, 41 min
50	0	—

TABLE III
Comparison of dose, mortality, and time to death for male Sprague-Dawley rats exposed to DBNP in corn oil

Dose (mg/kg)	Mortality (%)	Time to death (maximum)
250	100	3 hr, 26 min
98	60	22 hr, 25 min
78	20	7 hr, 13 min
62.5	0	—

Within the 78 mg/kg dosing group mortality was 20 percent (1 of 5). The animal died within 7.25 hours of dosing and had a peak body temperature of 108°F prior to death. Peak body temperature in the surviving animals was 105°F, seen at 7.5 hours post-dosing. All of the surviving animals returned to normal body temperature within 24 hours post-dosing. Prostration, rapid breathing, and rigor were evident in all of the animals.

All of the animals dosed at 62.5 mg/kg survived the 14 day post-dosing observation period. Hyperthermia, prostration, rigor, and rapid breathing were evident in this group, similar to findings in the higher dose groups. Table III provides information comparing dose level to mortality and time to death among the male SD animals.

Five male SD rats were exposed to DBNP at 98 mg/kg using carboxymethylcellulose as the vehicle. Mortality was 20 percent. Rigor, prostration, hyperthermia, and rapid breathing were evident in the exposed animals.

There was no mortality within the control animal groups. Weight loss was found immediately post-dosing, but the recovery time was rapid and the animals returned to normal weight gains within the 14-day observation period. Histologic evaluation indicated that there were no gross or microscopic lesions in the control animals.

In both studies, the exposed and control animals had some weight loss immediately after dosing. In the exposed groups the weight loss was marked and the surviving animals did not return to normal weight, or daily weight gain, during the 14-day observation period. In the control group, the weight loss occurred in the period immediately after dosing and was of short duration, and the animals returned to normal weight and weight gain within the observation period. Prostration, rigor, rapid breathing, and hyperthermia were notable in all the dosed animals, but not present among the controls.

Findings of the dermal absorption of DBNP in rabbits indicated that the material is not readily absorbed through intact skin. The concentration of DBNP in the blood and organs of the animals was below the limit of detection and determination of a

transfer coefficient was not made. No signs of toxic effects were noted in the animals during the 14-day observation period. None of the test animals had gross lesions noted at necropsy. Histologic examination from the treated skin areas of all five rabbits showed no abnormalities. Among the test animals 40 percent had statistically significant changes in weight gain from controls. In humans, dermal contact and yellowing of the skin could contribute to the oral ingestion dose if good personal hygiene practices are not followed. This route of exposure requires further evaluation.

DISCUSSION

DBNP is developed when DBP passes through a clean ESP and is nitrated. EBD found that DBNP generation is more strongly correlated with the generation in the oxides of nitrogen than with DBP concentration in the oil. Efficiency of the ESP decreases with the time of operation since the last cleaning. Oxides of nitrogen (N_{ox}) and DBNP show increased generation rates when the unit is working at peak efficiency and un-reacted oil mist passage through the ESP is at a minimum. As the unit operates and the plates become covered with particles and other materials the efficiency of operation decreases, the passage of un-reacted oil increases, and the generation of DBNP and N_{ox} decreases.

With regard to the thermogenesis and rigidity in the animals exposed to DBNP, it is probable that DBNP is similar to 2,4-Dinitrophenol (DNP), acting to uncouple oxidative phosphorylation.[7] Heat generated from the shift away from ATP production is used in some plants and in hibernating animals to maintain body temperature. The mechanism of this process is well recognized.[8] DBP and 2,6-Di-*tert*-butyl-Methylphenol (DBMP), both found in the oils and in the oil mist aerosols, do not have a similar mechanism of action. This dissimilarity is most probably due to the lack of a nitro group in DBP and DBMP. Rivera-Nevares et al. reported that DBNP was second only to 3,5-di-tert-butyl-4-hydroxybenzyliden e malononitrile in its action as an ATP uncoupler, based on quantum-mechanical calculations.[9] The effects of chronic exposures to DBNP at low concentrations will require further research.

Changes in the strain of rat did not appear to affect the LD_{50} value. Changing the vehicle from corn oil to carboymethylcellulose significantly changed the mortality rate in this study, however the change was based on a limited number of animals ($n = 5$) and requires further evaluation. The change in vehicle could explain a portion of the difference between the results of this study and the 1961 LD_{50} determination. Prostration, rigidity in the body, and hyperthermia were evidenced within all animals in both acute oral studies. Even the lowest dosage in the acute oral studies, 50 mg/kg, elicited these responses indicating that the lowest observable adverse effect Level (LOAEL) is < 50 mg/kg in rats. Within an hour of the dosing, the animal's body temperature began to rise. Elevated temperatures ranged from 105°F to 109.1°F. Rigor and rigidity in the animals was noteworthy. Animals died within an hour of the temperature spike or returned to normal and survived the exposure. Histopathology findings were similar in both acute oral studies. Noteworthy lesions included degenerative changes in skeletal, smooth, and cardiac muscle. Hepatocellular changes were also seen. The clinical observations were consistent with the assessments of the microscopic lesions suggesting metabolic changes which quickly led to cardiac failure and death.

The distribution of DBNP throughout the ventilation system on submarines, in surface ships, and in refineries could lead to potential exposures through dermal absorption, oral ingestion, and inhalation of the DBNP crystals. While results of the dermal uptake study in rabbits indicate that DBNP does not appear to be readily absorbed through intact skin, poor personal hygiene could contribute to ingestion of additional DBNP. EBD found that the DBNP precipitates in flakes or splinters which could effect settling rates and aerodynamic properties. Information about several factors, including density and particle dynamic shape factor, would be necessary to model the distribution of the DBNP through the ventilation systems and internal compartments of the submarine. EBD did not spike the lubricating oil with DBNP to determine capture efficiency in their sampling procedure. This information could also have been helpful in determining the potential (expected) inhalation dose that crew members might receive during underway periods of 24 hrs/day for 90 days.

Based on the results of these studies, a log-probit analysis was conducted to determine the LD_{50} for the male Fisher-344 and SD rats using Slidewrite software. Figures 4 and 5 provide the graphical presentation of this analysis. In the Fisher-344 study, the intent was to validate the LD_{50} in the range of 500 mg/kg. All the dosings occurred simultaneously so that the heightened mortality led to analytical concerns. With 100 percent mortality in the two highest dosing groups and 0 percent mortality in the lowest dose group, only one data point was available to predict the LD_{50}. The data point at 500 mg/kg was dropped from the analysis. The LD_{50} value for DBNP in corn oil for male Fisher-344 rats was found to be 80 mg/kg (slope $= 0.44$, $R^2 = 0.95$). The slope of the best-fit line for the log-probit plot is 0.47, but actually ranges from that value to undefined.

In the Sprague-Dawley rats the LD_{50} was found to be 80 mg/kg (slope $= 0.53$, $R^2 = 0.91$). The slope of the best-fit line overall was 0.53. Finally, the slope of the best-fit line and line segment of the actual data points between 20 percent and 60 percent mortality at dose levels of 78 mg/kg and 98 mg/kg, respectively, were equal, indicating that the lines were parallel but offset in the area of the LD_{50}. The LD_{50} derived from the best-fit line was 100 mg/kg.

The Navy's Bureau of Medicine and Surgery (BUMED) has been attempting to set an allowable exposure limit for DBNP in submarine environments to protect the health of the crew. A reference dose (RfD) calculation is not possible since the no observable adverse effect level (NOAEL) was not determined, however using the standard model format[10] the RfD should be

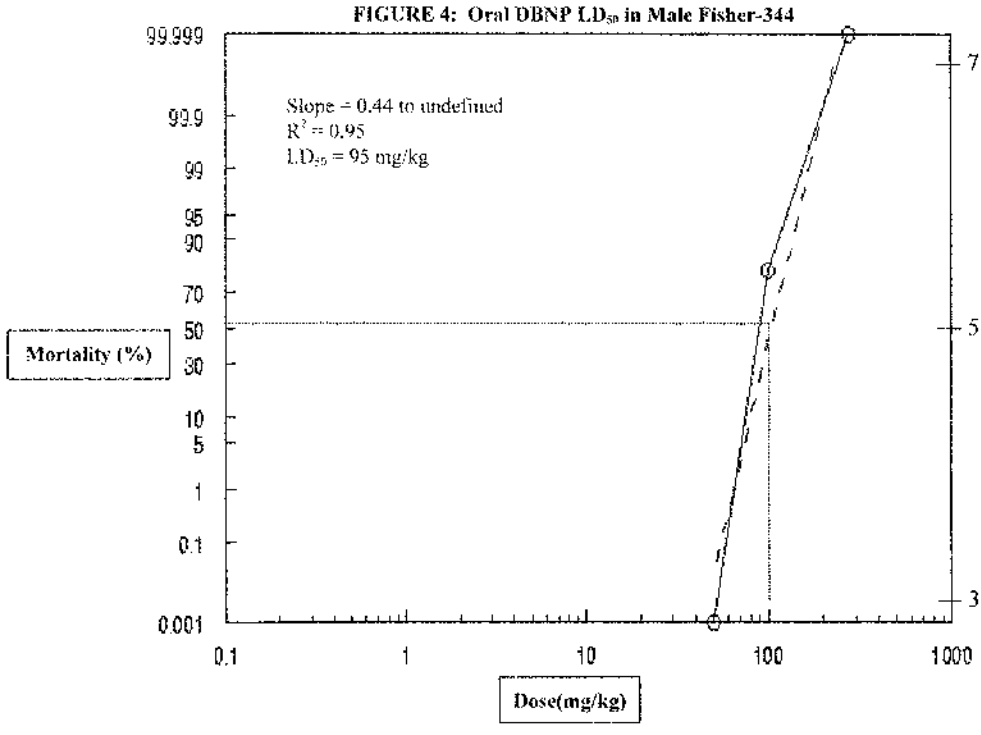

FIGURE 4
DBNP oral LD_{50} in male fisher-344 rats.

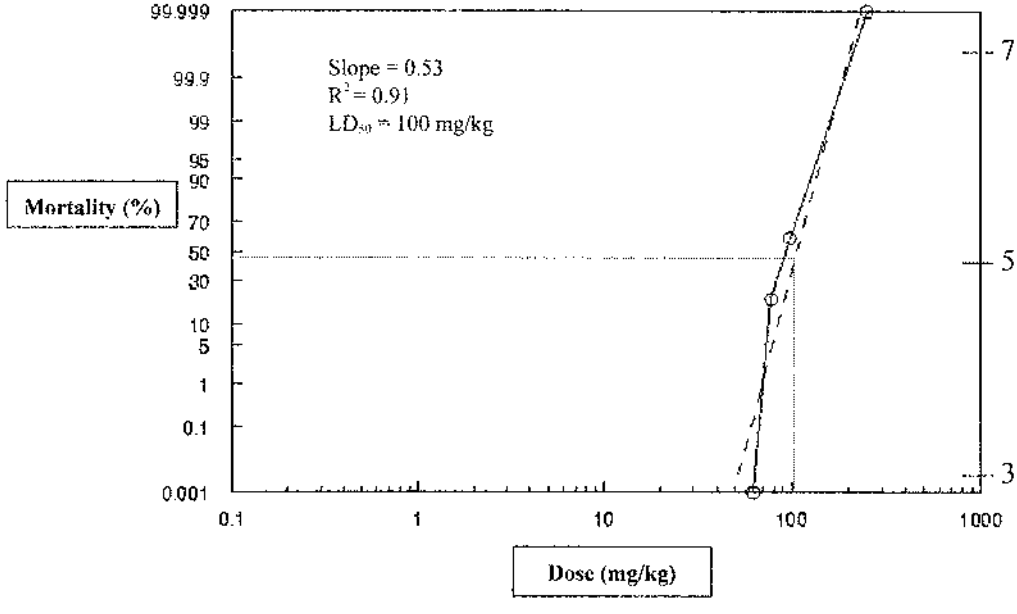

FIGURE 5
DBNP oral LD_{50} in male Sprague-Dawley rats.

calculated as:

$$RfD = NOAEL/UF \times MF$$

Where:

RfD is the reference dose
NOAEL is the no observable adverse effect level dosage
UF is the product of the uncertainty factors
MF is the product of the modifying factors

Without the NOAEL, LOAEL could be used for RfD calculation with adjustments in safety factors (uncertainty factors and modifying factors); however, no LOAEL was determined in this study and the effects of exposure (elevated temperature, rigor, and lethargy) were obvious even at the lowest dose levels.

In this project four uncertainty factors (UF) were identified including:

1. UF1 = to account for the differences between normal and sensitive populations.
2. UF2 = to account for species differences between rodents and humans.
3. UF3 = to account for differences in acute versus chronic exposures.
4. UF4 = to account for use of an LD_{50} versus a NOAEL.

Additionally a modifying factor (MF) has been identified and included:

- MF1 = for professional judgement and inadequacies in existing data.

To provide a conservative estimate and maintain the health and safety of personnel, the value for each UF and MF was set at 10, yielding an overall factor of 1×10^5. This value is two orders of magnitude greater than those recommended in texts. Maximizing the identified uncertainty factors will allow for additional concerns that have been recognized, but were not included in the UF/MF list, including 24-hour exposures for as long as 90 days in the closed environment of a submarine.

Using the LD_{50} of 80 mg/kg, in lieu of a NOAEL/LOAEL, and a 70 kg individual breathing 20 m^3 of air with UF and MF values of 1000 (recommended maximum value) and 100,000 based on identified factors, the maximum allowable exposure concentration (in air) is estimated at 0.28 to 0.0028 mg/m^3. These values, in parts per billion for direct comparison to EBD data quantifying airborne concentrations, range from 27.3 to 0.273 ppb,[11–12] respectively. The EBD data indicated airborne concentrations ranging from <3.0 ppb to 122 ppb and did not account for ingestion or dermal absorption in the total uptake. Using the lowest exposure value of 50 mg/kg, the allowable airborne concentration range falls to 0.18 to 0.0018 mg/m^3 or 17.53 to 0.175 ppb, respectively.

Further research is necessary to clarify the risk associated with exposure to DBNP. This research should include a complete clarification of the aerosol generation, transport, and deposition processes. Clarification of the exposure/dose/response relationships is necessary for each identified route of exposure in acute, sub-chronic, and chronic exposure scenarios. Determination of the mechanisms of action on organ systems, specifically the reproductive and nervous systems, should be conducted to clarify the toxicity of DBNP. Continued use of DBP-containing oils in various occupational settings indicates that individuals could be exposed to DBNP on a daily basis. Without completion of this research, human health risk assessments will be incomplete.

CONCLUSIONS

The health risk that DBNP poses to exposed workers is unresolved. While dermal absorption efficiency is limited in intact skin, the aerosol of DBNP can move through ventilation systems to other areas in a submarine, ship, or industrial plant. Initially, this poses a potential for exposure through inhalation. An adequate model of DBNP generation, transport, deposition, and respiratory effects does not exist. In other areas including food preparation/service locations, break areas, or living spaces, ingestion hazards may also exist. Individuals with DBNP on their skin, with or without yellowing, may ingest additional DBNP, increasing the dosage through this route of exposure. DBNP was found to be relatively non-toxic in the literature, with an LD_{50} in the range of 500 mg/kg. Through this project, DBNP has been shown to be more toxic than originally reported.

ACKNOWLEDGMENTS

Funding for this project was provided by the Office of Naval Research, the Navy Environmental Health Center, and Mobil Technology Corp. The authors acknowledge R. L. Carpenter, H. Zhang, A. Jung, S. Prues, and N. Loy of NHRC/TD for their participation and assistance in this project. We also thank personnel from the Air Force Research Laboratory Human Effectiveness Directorate (AFRL/HEST), including R. J. Godfrey, for their technical assistance in the conduct of this research project.

STATEMENT AND DISCLAIMER

The experiments reported herein were conducted according to the principles set forth in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, DHHS, Publication No. (NIH) 86–23 (1996).

The opinions and assertions contained herein are those of the authors and are not to be construed official or as reflecting the views of the Department of the Navy or the Naval Service at large, nor of other government agencies. This research was supported by the Naval Medical Research and Development Command, Department of the Navy Research Task NEHC 1323.

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