

# **APPENDIX E:**

## **SMOKE AND MASKING AGENTS**



## ADVERSE HEALTH EFFECTS FROM HEXACHLOROETHANE SMOKE

### General Reviews / Comments

The US National Research Council produced a report on the toxicity of military smokes and obscurants.<sup>1</sup> The first volume (1997) dealt with four obscurant smokes; diesel fuel, fog oil, red phosphorus and hexachloroethane. Concerns expressed about the health effects of hexachloroethane smoke included: nausea, cough, irritation of the nose, throat and chest, chemical pneumonia, pulmonary oedema, acute respiratory distress syndrome and death in humans. Alveolar carcinoma in mice was found in one study. Relevant sections of this report are reproduced as follows:

*Hexachloroethane (HCE) smoke (often referred to as HC smoke) is produced by burning a mixture containing roughly equal parts of HCE and zinc oxide and approximately 6% granular aluminum. The toxicity of HC smoke is attributed to the production of zinc chloride (ZnCl<sub>2</sub>).*

*Most reports of accidental human exposures to HC smoke indicate symptoms consistent with exposures to the ZnCl<sub>2</sub> component released when the smoke bomb is ignited.*

*Because of ZnCl<sub>2</sub>'s affinity for water, the aerosol likely consists of the hydrated forms of ZnCl<sub>2</sub> under most atmospheric conditions (Katz et al 1980). A starter mixture containing silicon, potassium nitrate, charcoal, iron oxide, granular aluminum, cellulose nitrate, and acetone, which is required to ignite the reaction, might generate very small amounts of other airborne contaminants. However, the acute toxic effects of exposure to HC smoke are considered to arise primarily from inhalation of the ZnCl<sub>2</sub> component, which comprises almost two thirds of the total mass of HC smoke (Table 5-2: zinc chloride 62.5%, zinc oxide 9.6%, iron oxide 10.7%, aluminium oxide 5.4%, lead oxide 1.0%, total particulate phase 89.2%, chlorinated vapours 10.8%).*

*The munitions listed in Table 5-1 all use slightly different chemical mixtures (Novak et al 1987). An analysis of trace materials in HC smoke mixtures found common zinc impurities (Katz et al 1980). Arsenic ranged from 0.13 to 5.0 microgram per gram (ug/g), mercury from 0.35 to 0.60 ug/g, cadmium from 53 to 1,523 ug/g, and lead from 50 to 858 ug/g.*

*The most extensive field study of HC smoke, reported by DeVaul et al (1989), shows the average smoke composition observed over five experiments. Composition and sampling location varied from test to test. In these tests, the total weight of smoke released ranged from 218.5 to 229.3 kg for groups of 18 to 20 M5 smoke pots. The particle mass-median diameters ranged from 0.77 to 1.05 um, with geometric standard deviations from 1.78 to 2.36. Those particle sizes generally agree with those found by Katz et al (1980) for aged aerosol and by Young et al (1989), confirming that the aerosol has a large respirable mass*

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<sup>1</sup> National Research Council (1997). Toxicity of Military Smokes and Obscurants. Volume 1. Subcommittee on Military Smokes and Obscurants. National Academy Press: Washington, DC. Accessed at <http://www.nap.edu/openbook/0309057361/html>

*fraction. DeVaul et al (1989) also measured four specific chlorinated organic compounds. The geometric mean ratios of tetrachloromethane, tetrachloroethylene, hexachloroethane, and hexachlorobenzene to zinc in HC smoke were found to be 0.014, 0.009, 0.010, and 0.030, respectively.*

*In addition to exposure to the smoke itself, workers manufacturing HC smoke munitions, might be exposed to toxic materials. The most significant possible exposure is to HCE. HCE is a white crystalline solid with a vapour pressure equivalent to 770 ppm at 25 degrees C (Eaton et al 1994). Its camphor like odor can be detected as low as 0.15 ppm. The HCE concentration in HC-smoke munitions production areas was found to be above the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value (TLV) of 9.7 mg per cubic metre (Selden et al 1993). Workers in these areas are protected with either air-supplied hoods at fixed work stations or full-face-piece respirators with both filters and organic vapor cartridges. Nevertheless, the plasma concentrations of HCE in workers rose from 0.08 (+ or - 0.14) ug/L to 7.30 (+ or - 6.04) ug/L after working for more than five weeks in the loading and packing operations (Selden et al 1993).*

*Inhalation is expected to be the most important route of exposure. Undoubtedly excessive exposure has occurred in the military. Hill and Wasti (1978) summarised case reports of accidental exposures, many of which were fatal. The fatal exposures resulted from the discharge of HC smoke devices in enclosed spaces. The exposures in these reported cases generally are poorly characterized and represent only the most extreme conditions.*

*Death can occur with exposures to  $ZnCl_2$  and has been attributed to respiratory insufficiency due to edema of the lungs or acute respiratory distress syndrome. The lethal dose of  $ZnCl_2$  in humans has been estimated to be 50,000 mg~min/cubic metre, an exposure that could be achieved according to Cullumbine (1957) by one generator in a 100 cubic foot room within 2 to 3 min. Death is usually delayed by several days. For those who survive an acute exposure, recovery can be protracted. Inhalation of HC smoke causes respiratory effects in humans and animals.*

*Pulmonary effects [in humans] include dyspnea, chest constriction, retrosternal and epigastric pain, hoarseness, cough, lacrimation, expectoration, and occasional hemoptysis. Sequelae can include cyanosis, elevated pulse, fever, and widespread edema. Exposure to low concentrations results in moderate presentation of these symptoms (Donohue et al 1992). Cullumbine (1957) reported that concentrations of  $ZnCl_2$  at 80 mg/cubic metre for 2 min (160 mg~min/m<sup>3</sup>) produced slight nausea and cough, and 120 mg/cubic metre for 2 min (240 mg~min/m<sup>3</sup>) resulted in irritation of the nose, throat, and chest; cough; nausea.*

*With CT products at 1,700 mg~min/m<sup>3</sup> and above, effects can be severe and require hospitalization and treatment.*

*The lower limit of detection of HC smoke by humans is reported to be approximately 40 mg/cubic metre (Schenker et al 1981). In an accidental exposure case, pneumonitis was reported in teens after exposure to  $ZnCl_2$  smoke produced*

by a grenade at a concentration of 4,075 mg/cubic metre (Johnson and Stonehill 1961). The exposed individuals experienced nausea and other respiratory symptoms, such as those mentioned above. Table 5-5 summarizes the effects of inhaled  $\text{ZnCl}_2$  smoke at various combinations of concentration and time.

$\text{ZnCl}_2$  [in humans] is corrosive and astringent and known to cause burning of moist body surfaces, including the respiratory and gastrointestinal tract. It has been reported to damage nerve endings in the nasal passages and to cause eye burns, damaging smell and vision. The upper respiratory tract is most affected by exposure (ATSDR 1994).

Acute oral exposures of humans to  $\text{ZnCl}_2$  are associated with numerous symptoms that include vomiting, diarrhea, lethargy, and irritation of the mouth, throat and stomach. Ingestion can produce corrosive gastritis and liver necrosis. In one case of food poisoning (83 mg of Zn per 100g of apples), characteristic symptoms were salivation; edema of the glottis; difficulty swallowing and massive swelling of the lips; pain in the mouth, throat, and epigastrium; recurrent intense vomiting; severe abdominal pain; and bloody diarrhea. Concentrations of 225 to 450 mg are known to be emetic. Two incidents of mass food poisoning produced symptoms that included abdominal cramping and occasional nausea and vomiting (ATSDR 1994).

Virtually no data are available at present on the effects of repeated exposure of neurological, reproductive, developmental, or immunological effects of  $\text{ZnCl}_2$  in humans.

In the worst-case scenario [for a Military Operation On Urban Terrain or MOUT], that concentration would result in exposures as high as 225 mg~min/cubic metre [of  $\text{ZnCl}_2$ ] (ie 1 mg/cubic metre \* 225 min). Thus, the current MOUT exercises might engender  $\text{ZnCl}_2$  exposures that exceed the human threshold for adverse respiratory-tract effects at 160 to 240 mg~min/cubic metre. Additionally, the values from the simulated MOUT exercise do not specify distance from the source of the smoke pot or the direction of air movement with respect to the source and exposed personnel. As indicated in Table 5-4, moderate distances (ie 40 to 190 m) downwind of a smoke pot might result in exposure concentrations of 2,000 mg~min/cubic metre or higher, which could result in the need for hospitalization and treatment.

Data from animals are sparse but indicate a NOAEL for HC smoke with  $\text{ZnCl}_2$  at 26.6 mg/m<sup>3</sup> in rodents for daily 1-hr exposures and a LOAEL for HC smoke with  $\text{ZnCl}_2$  at 254 mg/m<sup>3</sup> for inflammatory changes in the lung and death, suggesting a relatively steep dose response curve.

HC smoke has been reported to produce alveolar carcinomas in mice. Fitting a generalized multistage linear dose response model to those data provides an upper limit of the cancer risk of 0.086 per milligram  $\text{ZnCl}_2$  per kilogram of body weight per day.

The major histopathological finding of Marrs et al (1988) was an increase in the incidence of alveologenic carcinomas in mice exposed to the highest air concentration [1 hr per day, 5 days per week, for 20 weeks, for a total of 100 1-hr

exposures] (ie Zn at 122 mg/cubic metre, or ZnCl<sub>2</sub> at 254 mg/cubic metre, assuming that all Zn is in the form of ZnCl<sub>2</sub>).<sup>2</sup> International Agency for Research on Cancer (IARC) has not evaluated ZnCl<sub>2</sub> for its carcinogenicity. Genotoxicity studies in bacterial and mammalian-cell culture test systems provide no evidence that ZnCl<sub>2</sub> is mutagenic (ATSDR 1994).

**TABLE 5-5 Effects of Inhaled ZnCl<sub>2</sub>**

Concentration x Time (mg•min/m <sup>3</sup> )	Effect
<160	Essentially no effect; some awareness of presence
160-240	Noticeable irritation of nose, throat, and chest
1,700-2,000	Marked irritation: Hospitalization and treatment required
20,000	Severe irritation; chemical pneumonia: Hospitalization and treatment required
50,000	Massive injury; fatality

Sources: Storum and Hamilton (1976) as cited in Donohue et al. (1992).

<sup>2</sup> Marr T, Colgrave H, Edginton J, et al (1988). The repeated dose toxicity of a zinc oxide / hexachloroethane smoke. Arch Toxicol 62: 123-132. ID 27771

The US Agency for Toxic Substances and Disease Registry (1994) produced a toxicological profile for zinc.<sup>3</sup> Relevant sections relating to zinc chloride are reproduced as follows:

#### *Death*

*In humans, death has resulted from acute exposure to zinc compounds. When a high concentration (estimated at 33,000 mg zinc/m<sup>3</sup>) of zinc chloride smoke resulted from the explosion of many generators in a tunnel following a bombing raid in World War II, 10 of the 70 exposed people in the tunnel died within 4 days (Evans 1945). The smoke generated contained mainly zinc chloride, but exposure to other constituents, namely zinc oxide, hexachloroethane, calcium silicate, and an igniter, was also possible. Therefore, the deaths resulting from the explosion cannot be conclusively attributed to exposure to zinc chloride only. This is the only human study reporting an estimated exposure level that caused death. Hence, this level is reported as a LOAEL in Table 2-1 and Figure 2-1. Another study reported the death of a fireman exposed to the contents of a smoke bomb in a closed environment (Milliken et al. 1963). The man died 18 days after exposure because of respiratory difficulty. Again, exposure to zinc chloride was simultaneous with exposure to other substances in the smoke. Two soldiers exposed without gas masks to zinc chloride smoke during military training developed severe adult respiratory distress syndrome (ARDS) and died 25-32 days after the incident (Hjortso et al. 1988). Diffuse microvascular obliteration, widespread occlusion of the pulmonary arteries, and extensive interstitial and intra-alveolar fibrosis were observed at autopsy. Zinc levels in major organs and tissues obtained during autopsy were within the normal range, and no zinc particles were observed by scanning electron microscopy. According to the authors, the fumes from the smoke bombs consisted mainly of zinc chloride. However, no exposure levels were estimated, and other substances were also present in the smoke.*

*In mice, the reported LCT50, (product of lethal concentration and time to kill 50% of animals) of zinc chloride is 11,800 mg-min/m<sup>3</sup> (Schenker et al. 1981). However, Schenker et al. (1981) did not provide information on how this value was determined. Following exposure to zinc chloride smoke for 3-20 weeks, mortality was 50% in mice exposed to 121.7 mg zinc/m<sup>3</sup> (compared to 20% in controls) and 22% in guinea pigs exposed to 119.3 mg zinc/m<sup>3</sup> (compared to 8% in controls) (Marrs et al. 1988). The smoke was similar to that described by Evans (1945) and also contained zinc oxide, hexachloroethane, and other compounds.*

#### *Respiratory Effects*

*Zinc chloride, a corrosive inorganic salt, is more damaging than zinc oxide to the mucous membranes of the nasopharynx and respiratory tract upon contact. Zinc chloride is a primary ingredient in smoke bombs used by the military for screening purposes, crowd dispersal, and occasionally in military and civilian fire-fighting exercises. Reports of serious respiratory injury have been reported to result from accidental inhalation of smoke from these bombs. These reports are of limited use in assessing the toxicity of zinc chloride because exposure to other compounds,*

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<sup>3</sup> ATSDR (1994). Toxicological Profile for Zinc. Accessed at <http://www.atsdr.cdc.gov/toxprofiles/tp60.html>

usually hexachloroethane, zinc oxide, and calcium silicides, also occur. Furthermore, the specific concentrations inhaled are usually unknown. Despite these limitations, several case studies have described similar respiratory effects in humans following acute inhalation exposures. These effects include dyspnea, cough, pleuritic chest pain, bilateral diffuse infiltrations, pneumothorax, and acute pneumonitis from respiratory tract irritation (Johnson and Stonehill 1961; Matarese and Matthews 1966; Schenker et al. 1981). In the study by Johnson and Stonehill (1961) cough, dyspnea, burning throat, diffuse infiltrates throughout the lung, chemical pneumonitis, and decreased vital capacity were observed at an estimated zinc chloride exposure level of 4,075 mg/m<sup>3</sup> (1,955 mg zinc/m<sup>3</sup>). In other studies, more severe effects have occurred, including ulcerative and edematous changes in mucous membranes, fibrosis, subpleural hemorrhage, advanced pulmonary fibrosis, and fatal respiratory distress syndrome (Evans 1945; Hjortso et al. 1988; Homma et al. 1992; Milliken et al. 1963).

Focal alveolitis, consolidation, emphysema, infiltration with macrophages, and fibrosis were observed in guinea pigs that died following exposure to 119 mg zinc/m<sup>3</sup> as zinc chloride smoke for 1 hour/day, 5 days/week, for up to 3 weeks (Marrs et al. 1988). Thirteen months after a 20-week exposure, rats and mice inhaling 121.7 mg zinc/m<sup>3</sup> as zinc chloride smoke for 1 hour/day, 5 days/week, showed increased macrophages in the lungs (Marrs et al. 1988). The smoke also contained zinc oxide, hexachloroethane, and other compounds.

#### *Gastrointestinal Effects*

Nausea was reported by humans exposed to high concentrations of zinc chloride smoke (Evans 1945; Johnson and Stonehill 1961; Schenker et al. 1981). The zinc chloride smoke also contained zinc oxide, hexachloroethane, and other compounds. In general, exposure levels associated with nausea have not been reported. Autopsies of victims who died following exposure to very high concentrations of zinc chloride smoke revealed irritation of the stomach and intestines (Evans 1945). The smoke also contained zinc oxide, hexachloroethane, and other compounds.

The only information available regarding gastrointestinal effects in animals was found in a study by Marrs et al. (1988) in which rats and mice were exposed to 121.7 mg zinc/m<sup>3</sup> as zinc chloride smoke (which also contains zinc oxide, hexachlorophene, and other compounds) for 1 hour/day, 5 days/week, for 20 weeks, and then observed for an additional 13 months. In the same study, guinea pigs were exposed to 119.3 mg zinc/m<sup>3</sup> as zinc chloride smoke for 1 hour/day, 5 days/week, for 3 weeks. All animals were sacrificed at the end of 18 months. Routine gross and microscopic evaluation of the stomach and intestines at 18 months revealed no persistent adverse effects.

Several studies have suggested that zinc ingestion may cause symptoms of gastrointestinal distress or alterations in gastrointestinal tissues. For example, one individual who ingested about 3 ounces of a zinc chloride solution described acute symptoms that occurred almost immediately following contact with the compound, including burning and pain in the mouth and throat and vomiting (Chobanian 1981). Later, the patient exhibited pharyngitis, esophagitis, hypocalcemia, and



*elevated levels of amylase; the latter two alterations are suggestive of acute pancreatitis. The patient received intravenous hydration and calcium supplementation and recovered within 5 days. The material ingested was described as a "zinc chloride solution," and its concentration was not reported. Therefore, a dose level could not be established in this case.*

#### *Dermal / Ocular Effects*

*In a case report, accidental splashing of a soldering paste containing 30% zinc chloride into the eye of a plumber produced an immediate reduction in visual acuity, hyperemia, hemorrhaging, conjunctival swelling, corneal opacity, bullous keratopathy, and spotting of the lens (Houle and Grant 1973). Most symptoms disappeared after 6 weeks, but residual lens opacities persisted for over a year after the exposure.*

*Reddened conjunctivae and lacrimation were observed in 34 persons who were exposed to extremely high concentrations of zinc chloride smoke when several smoke generators exploded in a tunnel during World War II (Evans 1945). Two of the exposed persons had corneal burns and four had small vesicular burns on the forehead or wrist. Zinc chloride was the major component of the smoke. However, other components such as zinc oxide, hexachloroethane, calcium silicide, the igniter, or the heat of the explosion may have contributed to the injuries that were observed.*

*The dermal irritancy of several zinc compounds was compared in mice, rabbits, and guinea pigs (Lansdown 1991). Of the six zinc compounds tested, zinc chloride had the greatest irritancy potential, followed by zinc acetate and zinc sulfate; no signs of irritation were observed following exposure to zinc oxide. Although zinc chloride is clearly the most irritating, the relative irritancy of zinc sulfate and zinc acetate was not determined because only one dose was tested and a different dose was used for each compound. The severe skin irritancy observed following application of zinc chloride was characterized by parakeratosis, hyperkeratosis, inflammatory changes in the epidermis and superficial dermis, and acanthosis of the follicular epithelia (Lansdown 1991).*

#### *Hematological Effects*

*A worker who had been employed making up zinc chloride solutions (concentrations not specified) with his hands [dermal exposure] was found to have microcytic anemia and decreased numbers of platelets (DuBray 1937).*

*In animals, following oral administration of zinc compounds, decreased hemoglobin, hematocrit, erythrocyte, and/or leukocyte levels were observed in rats (Maita et al. 1981; Smith and Larson 1946), mice (Maita et al. 1981; Walters and Roe 1965) rabbits (Bentley and Grubb 1991) dogs (Drinker et al. 1927d; Meurs et al. 1991; Robinson et al. 1991), ferrets (Straube et al. 1980), and preruminant calves (Jenkins and Hidioglou 1991). In rats, the lowest LOAEL for decreased hemoglobin (85% of control value) is 12 mg zinc/kg/day as zinc chloride in a 4-week drinking water study with 2-month-old rats (Zaporowska and Wasilewski 1992).*

### *Reproductive Effects*

*The frequency of sperm with an altered chromatin structure was increased in rats fed 25 mg zinc/kg/day as zinc chloride for 8 weeks (Evenson et al. 1993).*

### *Developmental Effects*

*Single intraperitoneal injections of 12.5, 20.5, or 25 mg/kg zinc chloride on days 8, 9, 10, or 11 of gestation produced skeletal anomalies, including delayed ossification and rippled ribs without accompanying soft tissue defects in mice. Rippled ribs, an unusual anomaly, appeared when zinc salt was given on day 9 of gestation at a dose of 20.5 mg/kg, becoming more prevalent when 25 mg/kg of the salt was administered on day 11 of gestation (Chang et al. 1977).*

### *Genotoxic Effects*

*No studies were located regarding genotoxicity in humans after oral exposure to zinc.*

*Chromosomal aberrations were detected in the bone marrow cells of mice administered 350 mg zinc/kg as zinc chloride and fed a low-calcium diet (1.1% calcium), but not when the animals were given a similar zinc dose and fed a calcium-replete diet (Deknadt and Gerber 1979).*

*Genotoxicity studies conducted in a variety of test systems have failed to provide evidence for mutagenicity of zinc. However, there are indications of weak clastogenic effects following zinc exposure.*

*Results of in vivo studies are shown in Table 2-4. A dominant lethal study in mice failed to show a mutagenic potential for zinc. However, chromosomal aberrations have been observed in bone marrow cells following in vivo exposure to zinc (Vilkina et al. 1978). This effect was observed in rats exposed to 14.8 mg zinc/kg/day as zinc chlorate in drinking water (Kowalska-Wochna et al. 1980), mice given intraperitoneal injections of 3.6 mg zinc/kg/day as zinc chloride (Gupta et al. 1991), and mice exposed to zinc oxide by inhalation (Voroshilin et al. 1978). Chromosomal aberrations caused by zinc were observed in the bone marrow cells of mice maintained on a low-calcium diet (Deknadt and Gerber 1979). Calcium may be displaced by zinc in calcium-depleted conditions, leading to chromosome breaks and/or interfering in the repair process (Deknadt and Gerber 1979). An increased incidence of sister chromatid exchange was observed in bone marrow cells of rats exposed to 17.5 mg zinc/kg/day as zinc chlorate in drinking water (Kowalska-Wochna et al. 1988).*

*Results of in vitro studies are shown in Table 2-5. Exposure to zinc as zinc sulfate or zinc chloride does not increase mutation frequencies in bacterial or mammalian cell culture test systems (Amacher and Paillet 1980; Gocke et al. 1981; Marzin and Vo Phi 1985; Nishioka 1975; Thompson et al. 1989; Venitt and Levy 1974; Wong 1985). Similarly, there was no convincing evidence of a clastogenic effect in human lymphocytes exposed to 0.0003-0.00003 M zinc chloride (Deknadt and Deminatti 1978).*

## Cancer

*No reliable human carcinogenicity data were located.*

*Female Porton strain mice (98-100/group) exposed to 121.7 mg zinc/m<sup>3</sup> of a zinc oxide/hexachloroethane smoke mixture (which produces zinc chloride), 1 hour/day, 5 days/week, for 20 weeks had a statistically significant increase in the incidence of alveologenic carcinoma (30% versus 8% in control) thirteen months after the end of exposure (Marrs et al. 1988). No increased tumor incidences were seen in mice exposed to 1, 1.3, or 12.8 mg zinc/m<sup>3</sup>. Guinea pigs and rats were also tested with similar dose levels, and no significant carcinogenic response was observed. A number of factors limits the usefulness of this study, including the presence of several compounds in the smoke that may have carcinogenic potential, the use of only female animals, and the short duration of the exposure (20 weeks).*

The Agency for Toxic Substances and Disease Registry (1997) noted that while the smoke produced by burning a mixture of hexachloroethane (HCE) and zinc oxide (ZnO), so called HC smoke, is mainly zinc chloride, a minor component of the smoke is hexachloroethane.<sup>4</sup> About 5% or less of the reagents in a hexachloroethane containing smoke device is released to air as hexachloroethane in the smoke. Hexachloroethane in smoke (aerosol) was measured in a wind tunnel at concentrations ranging from 0.64 to 1.26 mg per cubic metre (Cataldo et al 1989). It was noted that military or civilian personnel working with smoke or pyrotechnic devices may be exposed to hexachloroethane. Relevant sections of this toxicological profile on hexachloroethane are reproduced as follows:

*Hexachloroethane is a solid crystalline material that has entered the environment as a result of its use in military pyrotechnics and as a component of smoke-producing devices used for screening or signaling purposes. It is an intermediate in the production of fluorocarbons, cleaning agents, and refrigerants and was once used in veterinary medicine to control liver flukes in sheep. It can be found at military disposal sites and at hazardous waste sites. In addition, hexachloroethane can be formed during incineration of chlorinated organic compounds and during chlorination of drinking water. Accordingly, there is some risk that humans can be exposed to this material.*

## Human Studies

*11 workers occupationally exposed to hexachloroethane at 0.5-2.1 ppm while wearing protective equipment including compressed-air-fed visors or full-facepiece masks with combination filters (Selden et al. 1994). The testing was completed 5 weeks after production at a smoke munitions plant resumed following a 5-week break. Plasma hexachloroethane levels were  $0.08 \pm 0.14$  µg/L before production resumed and  $7.3 \pm 6.04$  µg/L 5 weeks later indicating that despite protective equipment, low-level exposure occurred (Selden et al 1993). Hexachloroethane-exposed workers reported a slightly higher prevalence of dry skin and dry mucous membranes as well as itching and other skin problems than*

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<sup>4</sup> Agency for Toxic Substances and Disease Registry (1997). Toxicological Profile for Hexachloroethane. Accessed at <http://www.atsdr.cdc.gov/toxprofiles/tp97.html> On 16<sup>th</sup> July 2003.

*the unexposed controls (Selden et al. 1994). Clinical examinations of the 11 exposed workers did not reveal signs of abnormal dermatological status. The investigators indicate that the dermal effects may also have been a result of a local trauma effect of the protective equipment.*

*Respiratory, hematological, liver, and renal effects were not observed in 11 hexachloroethane-exposed workers.*

*Only one report was located regarding an association between hexachloroethane and cancer in humans (Selden et al. 1989). In this study a liver tumor was found in an adult male who had used a product containing hexachloroethane at work for 6 years. However, under the conditions of use, the hexachloroethane reacted to form hexachlorobenzene and other chlorinated compounds, which were as likely, or more likely, to have contributed to the tumorigenesis as the hexachloroethane.*

[No studies in humans following exposure to hexachloroethane were located regarding lethality, cardiovascular, gastrointestinal, musculoskeletal, endocrine, ocular, reproductive, developmental or genotoxic effects.]

#### *Animal Studies*

*Animal studies identify the kidney and liver as the primary target organs for hexachloroethane. Renal problems were most severe in male rats and were associated with alpha<sub>2</sub>μ-globulin hyaline droplet nephropathy. Minimal to mild lesions were also seen in female rat kidneys and in male and female mice, indicating that some mechanism, in addition to hyaline droplet formation, is involved in renal toxicity. The liver responds to hexachloroethane exposure with increases in liver weight, increases in serum levels of liver enzymes, centrilobular necrosis, fatty degeneration, hemosiderin-laden macrophages, and hemorrhage. Effects on the liver and kidneys were mild with inhalation exposure and more pronounced with oral exposure. No data were available for effects on the liver and kidneys by the dermal exposure route.*

*Hexachloroethane vapors and ingested hexachloroethane act as irritants on the lining of the lung, nasal cavity, trachea, and other tissues of the respiratory tract. Pulmonary irritation was associated with an increased incidence of mycoplasma infection in rats. Hexachloroethane exposure can also irritate the eyes. The irritation of the eye and respiratory tract are reversible once exposure has ceased. Both oral and inhalation exposures to high concentrations of hexachloroethane were associated with hyperactivity, ataxia, convulsions, and/or prostration in rats, sheep, and dogs. The mechanism for these neurological effects is not clear since there were no apparent histopathological lesions in the brains of the affected animals. Neurological effects were only noted with the high-dose exposures.*

*There has been no comprehensive evaluation of the reproductive and developmental effects of hexachloroethane. Limited data indicate that it is maternally toxic and retards fetal development. It does not appear to be a teratogen.*

The current U.S. Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for zinc chloride fume is 1 milligram per cubic meter of air as an 8-hour time-weighted average (TWA) concentration and 2 mg per cubic meter as a 15-minute TWA short-term exposure limit (STEL).<sup>5</sup> A STEL is the maximum 15-minute concentration to which workers may be exposed during any 15-minute period of the working day. The OSHA limits are based on the risk of respiratory irritation associated with exposure to zinc chloride fume. In the health hazard information section, it was noted that contact of the skin with zinc chloride dust can cause primary dermatitis and chemical burns (Rom 1983, page 505). Zinc chloride fume was considered a mutagen (HE2). HE2 stands for chronic (cumulative) toxicity - known or suspected animal or human carcinogen. This is in contrast to HE1 which, stands for cancer - currently regulated by OSHA as carcinogen.

The US Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) has established a permissible or recommended exposure limit for hexachloroethane of 10 mg per cubic meter of air as an eight-hour time-weighted average concentration.<sup>6 7</sup> Exposure to hexachloroethane can occur through inhalation, ingestion, eye or skin contact and absorption through the skin, eyes and mucous membranes. Acute exposure to hexachloroethane is moderately irritant to eyes, skin and mucous membranes in humans (OSHA, NIOSH). Excessive blinking, visual intolerance to light, tearing and reddened eyes have been reported in workers exposed to the vapours of hot hexachloroethane but no permanent damage to eyes was noted (OSHA). No signs or symptoms of chronic exposure to hexachloroethane have been reported in humans. In animals, eye and respiratory tract irritation, liver and kidney damage and central nervous system toxicity have been noted (OSHA, NIOSH). NIOSH considers that hexachloroethane is a potential occupational carcinogen.

The Chemwatch summary for hexachloroethane mentioned several potential risks based on limited evidence.<sup>8</sup> These included: possible cancer-causing agent, repeated exposure potentially causes skin dryness and cracking, and vapours potentially cause drowsiness and dizziness.

The Tenth Report on Carcinogens, by the US National Toxicology Program, did not list zinc chloride as an agent known to be a human carcinogen or reasonably anticipated to be a human carcinogen.<sup>9</sup>

The US National Toxicology Program (10th Report on Carcinogens) considers that hexachloroethane is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals.<sup>10</sup> When male rats were

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<sup>5</sup> Occupational Safety and Health Administration. US Department of Labor. Accessed at [www.osha.gov](http://www.osha.gov) on 17<sup>th</sup> June 2003. ID 27904.

<sup>6</sup> Occupational Safety and Health Administration. Chemical Sampling Information and Health Guidelines. Accessed at [www.osha.gov](http://www.osha.gov) on 21<sup>th</sup> July 2003.

<sup>7</sup> National Institute for Occupational Safety and Health. Pocket Guide to Chemical Hazards and International Chemical Safety Cards. Accessed at [www.cdc.gov/niosh](http://www.cdc.gov/niosh) on 21<sup>th</sup> July 2003

<sup>8</sup> Chemwatch. Summary for hexachloroethane. <http://dsmachem.dcb.defence.gov.au> on 15<sup>th</sup> May 2003

<sup>9</sup> US National Toxicology Program. Tenth Report on Carcinogens. US Department of Health and Human Services. Accessed at <http://ehp.niehs.nih.gov/roc/toc10.html> on 16<sup>th</sup> July 2003

<sup>10</sup> National Toxicology Program. US Department of Health and Human Services. 10<sup>th</sup> Report on Carcinogens. Accessed at <http://ehp.niehs.nih.gov> on 16<sup>th</sup> July 2003

administered hexachloroethane in corn oil by gavage, there was an increased incidence of renal neoplasms and a marginally increased incidence of adrenal pheochromocytoma. This was not seen in female rats. In similarly treated mice of both genders, there was a significant increase in the incidence of hepatocellular carcinoma. Little data was available in humans (IARC 1999).

The US National Toxicology Program (10th Report on Carcinogens) considers that hexachlorobenzene is reasonably anticipated to be a human carcinogen.<sup>11</sup> This was based on sufficient evidence of carcinogenicity in experimental animals (IARC 1982, 1987). When administered in the diet, hexachlorobenzene induced liver tumours in female rats and mice of both genders, and hepatomas, liver haemangioendotheliomas, and thyroid adenomas in hamsters of both genders (IARC 1979, Smith and Cabral 1980). There was inadequate evidence for the carcinogenicity of hexachlorobenzene in humans (IARC 1979, 1987).

The US National Toxicology Program (10th Report on Carcinogens) considers that carbon tetrachloride (ie tetrachloromethane) is reasonably anticipated to be a human carcinogen.<sup>12</sup> This was based on sufficient evidence of carcinogenicity in experimental animals. Sections of this Report are reproduced:

*Carbon tetrachloride (CCl<sub>4</sub>) is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals (IARC 1972, 1979, 1982, 1987, 1999). When administered by gavage, carbon tetrachloride increased the incidences of hepatomas and hepatocellular carcinomas in mice of both sexes. By the same route of administration, the compound increased the incidence of neoplastic nodules of the liver in rats of both sexes. When administered by subcutaneous injection, carbon tetrachloride induced hepatocellular carcinomas in male rats and mammary adenocarcinomas and fibroadenomas in female rats. When administered by inhalation, carbon tetrachloride induced liver carcinomas in rats. When administered intrarectally, the compound induced nodular hyperplasia of the liver in male mice.*

*No adequate data were available from human studies to evaluate the carcinogenicity of carbon tetrachloride in humans (IARC 1979, 1982, 1987, 1999). Three case reports described liver tumors associated with cirrhosis in humans exposed to carbon tetrachloride. A mortality study of laundry and dry cleaning workers exposed to a variety of solvents suggested an excess of respiratory cancers, liver tumors, and leukemia.*

The Tenth Report on Carcinogens by the US National Toxicology Program lists tetrachloroethylene as reasonably anticipated to be a human carcinogen based on limited evidence in humans and sufficient evidence in experimental animals for the carcinogenicity of tetrachloroethylene.<sup>13</sup> Sections of this Report are reproduced:

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<sup>11</sup> National Toxicology Program. US Department of Health and Human Services. 10<sup>th</sup> Report on Carcinogens. Accessed at <http://ehp.niehs.nih.gov> on 16<sup>th</sup> July 2003

<sup>12</sup> National Toxicology Program. US Department of Health and Human Services. 10<sup>th</sup> Report on Carcinogens. Accessed at <http://ehp.niehs.nih.gov> on 16<sup>th</sup> July 2003

<sup>13</sup> National Toxicology Program. US Department of Health and Human Services. 10<sup>th</sup> Report on Carcinogens. Accessed at <http://ehp.niehs.nih.gov> on 24<sup>th</sup> July 2003

*Tetrachloroethylene (perchloroethylene) is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals (NTP 1986, IARC 1979, 1987, 1995). When administered by inhalation, tetrachloroethylene increased the incidences of hepatocellular adenomas and carcinomas in male mice and hepatocellular carcinomas in female mice. By the same route of administration, the compound increased the incidences of mononuclear cell leukemia in rats of both sexes and rare renal tubular cell neoplasms in male rats. When administered by gavage, tetrachloroethylene increased the incidence of hepatocellular carcinomas in mice of both sexes.*

*There is limited evidence for the carcinogenicity of tetrachloroethylene in humans (IARC 1987, 1995). Tetrachloroethylene has been studied by observing laundry and dry-cleaning workers, who may also have been exposed to other solvents, especially trichloroethylene, but also petroleum solvents. In several cohort and proportionate mortality studies, excesses have been reported of lymphosarcomas, leukemias, and cancers of the skin, colon, lung, and urogenital tract. Some excess of lymphomas and of cancers of the larynx and urinary bladder was seen in a large cohort of dry cleaners. A familial cluster of chronic lymphocytic leukemia has also been related to dry cleaning. Although these studies suggest a possible association between long-term occupational exposure to tetrachloroethylene and increased lymphatic malignancies and urogenital cancers, the evidence must be regarded as inconclusive because workers were exposed to petroleum solvents and other dry cleaning agents as well as tetrachloroethylene. When all studies are considered, there is evidence for consistent positive associations between tetrachloroethylene exposure and esophageal and cervical cancer and non-Hodgkin's lymphoma. While these associations appear unlikely to be due to chance, confounding cannot be excluded; further, the total numbers in the cohort studies combined are relatively small (IARC 1995).*

*The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value (TLV) of 25 ppm (170 mg/m<sup>3</sup>) with a ceiling value not to exceed 100 ppm (685 mg/m<sup>3</sup>) for tetrachloroethylene. NIOSH has recommended that tetrachloroethylene be regarded as a potential occupational carcinogen. OSHA established a permissible exposure limit (PEL) of 25 ppm as an 8-hr time-weighted average (TWA) with no short-term exposure limit (STEL) or ceiling permitted. OSHA also regulates tetrachloroethylene under the Hazard Communication Standard and as a chemical hazard in laboratories.*

Hexachlorobutadiene was not listed in the US National Toxicology Program's Tenth Report on Carcinogens.<sup>14</sup>

Review of the database of the International Agency for Research on Cancer revealed no entry regarding an evaluation of the carcinogenicity of zinc chloride.

The International Agency for Research on Cancer (IARC 1999) has concluded that there is sufficient evidence in experimental animals but inadequate evidence in

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<sup>14</sup> National Toxicology Program. US Department of Health and Human Services. 10<sup>th</sup> Report on Carcinogens. Accessed at <http://ehp.niehs.nih.gov> on 16<sup>th</sup> July 2003



humans for the carcinogenicity of hexachloroethane.<sup>15</sup> The overall evaluation for hexachloroethane was possibly carcinogenic to humans (Group 2B). The summary is reproduced below.

*Exposure to hexachloroethane may occur during its production and use in metal refining, in fire suppression and in other minor uses.*

#### *5.2 Human carcinogenicity data*

*One cohort study of workers at aluminium foundries and aluminium smelters in Sweden showed no significant association between exposure to hexachloroethane and cancer incidence.*

#### *5.3 Animal carcinogenicity data*

*Hexachloroethane was tested in one experiment in mice and two experiments in rats by oral administration. It produced liver tumours in mice of each sex. In rats, it produced a statistically significantly increased incidence of renal tubular tumours in males in one study and a marginal increase in the incidence of renal tubular tumours in another study, also only in males. In a two-stage liver initiation-promotion assay in rats, hexachloroethane showed promoting but no initiating activity.*

#### *5.4 Other relevant data*

*No data were available to the Working Group on the absorption, distribution, metabolism or excretion of hexachloroethane in humans. It is absorbed in rats after oral administration, is concentrated in kidney and fat and is excreted by apparent first-order kinetics.*

*In humans, exposure by inhalation to hexachloroethane (10-20 mg/m<sup>3</sup>) produced mild irritation of the skin and mucous membrane. Inhalation produced respiratory irritation in rodents.*

*After short-term exposure, hexachloroethane caused renal toxicity in male rats and hepatocellular necrosis in both male and female rats.*

*The data on reproductive toxicity were inadequate for evaluation.*

*No data were available on the genetic and related effects of hexachloroethane in humans. Hexachloroethane was found to bind to DNA in mouse liver after intraperitoneal injection; no other data were available on its genetic effects in experimental systems in vivo. It induced sister chromatid exchange in one study but did not induce chromosomal damage in mammalian cells in vitro. It induced gene mutation in *Drosophila* and yeast but was not mutagenic to bacteria.*

#### *5.5 Evaluation*

*There is inadequate evidence in humans for the carcinogenicity of hexachloroethane.*

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<sup>15</sup> International Agency for Research on Cancer (1999). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 73: Some Chemicals That Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances. Lyon, France.



*There is sufficient evidence in experimental animals for the carcinogenicity of hexachloroethane.*

#### *Overall evaluation*

*Hexachloroethane is possibly carcinogenic to humans (Group 2B).*

IARC also commented on toxic effects in humans. It reported that Selden et al (1994) had studied the short-term adverse effects of hexachloroethane in a group of 11 munition workers who were exposed to hexachloroethane at concentrations of 10-20 mg per cubic metre or more for five weeks. A control group of age- and gender-matched unexposed subjects were used. The exposed group had mild skin and mucous membrane irritation but clinical and laboratory examinations revealed no adverse effects on blood, liver, kidney or lung function.

The International Agency for Research on Cancer (2001) concluded that hexachlorobenzene was possibly carcinogenic to humans (Group 2B) based on inadequate evidence in humans and sufficient evidence in experimental animals of carcinogenicity.<sup>16</sup> The summary evaluation is reproduced below.

*Hexachlorobenzene is a chlorinated hydrocarbon which may contain some higher polychlorinated dibenzofurans and dioxins as impurities. It has been used in the manufacture of industrial chemicals, including chlorinated pesticides, and as a fungicide and seed dressing in agriculture. The production and use of hexachlorobenzene have decreased since the 1970s owing to bans and restrictions on its use in many countries, but it still occurs as a by-product of the production of a number of chlorinated solvents and other industrial chemicals. Occupational exposure to hexachlorobenzene has occurred during its production and use in industry and agriculture. Hexachlorobenzene has been detected in many foodstuffs, but dietary intake has probably decreased in recent years.*

#### *5.2 Human carcinogenicity data*

*The risk for breast cancer has been investigated in relation to life-long, accumulated exposure to hexachlorobenzene in nine studies.*

*Five small case-control studies that included fewer than 50 cases of breast cancer each showed no overall association with the concentration of hexachlorobenzene in contemporary samples of adipose breast tissue. A secondary subgroup analysis in one of the studies revealed a significant association in postmenopausal women with estrogen receptor-positive cancer, based, however, on a small number of cases.*

*Four large case-control studies of exposure to hexachlorobenzene have been reported, one from Canada and three from the USA. In three of these, the concentration of hexachlorobenzene was measured in biological samples (serum fat or breast fat) from the study subjects, obtained close to the time of breast cancer diagnosis. No consistent increase in the risk for breast cancer was found in women with elevated concentrations of hexachlorobenzene. In the fourth case-*

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<sup>16</sup> International Agency for Research on Cancer (2001). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 79: Some Thyrotropic Agents. Lyon, France.

control study (from the USA), banked serum samples obtained before the breast cancer diagnosis were used to assess the body burden of hexachlorobenzene. The risk for breast cancer of women whose concentration of hexachlorobenzene was in the upper three quartiles was twice that of those whose samples were in the lower quartile. However, there was no evidence of a dose–response relationship, and the association was limited to women whose blood was collected close to the time of diagnosis of their breast cancer.

One case–control study each of endometrial cancer, pancreatic cancer and hairy-cell leukaemia yielded no notable results with respect to exposure to hexachlorobenzene.

### 5.3 Animal carcinogenicity data

Hexachlorobenzene was tested for carcinogenicity by oral administration in one study in mice, four studies in rats and one in hamsters. It produced liver-cell tumours in all three species and renal tubular tumours in rats of each sex in one study. After perinatal administration to rats, it increased the incidences of parathyroid adenomas in males and adrenal pheochromocytomas in females. In hamsters, it also produced liver haemangioendotheliomas and thyroid follicular-cell adenomas. In several studies in which it was given with other compounds, hexachlorobenzene promoted liver carcinogenesis in mice and rats.

### 5.4 Other relevant data

Hexachlorobenzene is lipophilic, accumulates in humans and is excreted as a cysteine conjugate of pentachlorobenzene. In rats, hexachlorobenzene has been shown to follow several metabolic pathways, which include the formation of pentachlorobenzene, tetrachlorobenzene and tri- and tetrachlorophenol.

Accidental consumption by humans of a large quantity of hexachlorobenzene resulted in porphyria cutanea tarda, liver toxicity, neurological effects and skin changes, which were persistent.

In experimental animals, the effects of treatment with hexachlorobenzene on the thyroid include decreased thyroid hormone concentrations due to increased glucuronidation and inhibition of type-I deiodinase, interference with serum carrier binding of the thyroid hormones and increased thyroid-stimulating hormone concentrations. In the livers of experimental animals, hexachlorobenzene induced cytochrome P450 enzymes and inhibited uroporphyrinogen decarboxylase, iron accumulation and oxidative damage. These effects are believed to be involved in the production of hepatic tumours.

In a poisoning epidemic in Turkey, exposure to hexachlorobenzene via breast milk caused a very high rate of lethality among infants. An increased frequency of pregnancy loss was reported among women exposed to hexachlorobenzene as children. The presence of this compound in breast milk has been associated with altered immune function in Inuits. Hexachlorobenzene was teratogenic in mice, and increased mortality rates were observed among rats and monkeys exposed in utero. Effects on steroid hormones have also been reported in exposed female mice.

In a single study of workers exposed to a number of chlorinated solvents, including hexachlorobenzene, an increased frequency of micronucleated lymphocytes was found; there was no association with the concentrations of hexachlorobenzene in

*blood. Micronuclei were induced by hexachlorobenzene in human and rat primary hepatocytes in vitro. Otherwise, there was little evidence that hexachlorobenzene has genetic activity.*

### *5.5 Evaluation*

*There is inadequate evidence in humans for the carcinogenicity of hexachlorobenzene.*

*There is sufficient evidence in experimental animals for the carcinogenicity of hexachlorobenzene.*

### *Overall evaluation*

*Hexachlorobenzene is possibly carcinogenic to humans (Group 2B).*

The International Agency for Research on Cancer (1999) concluded that tetrachloromethane (ie carbon tetrachloride) was possibly carcinogenic to humans (Group 2B) based on inadequate evidence in humans and sufficient evidence in experimental animals of carcinogenicity.<sup>17</sup> The summary evaluation is reproduced below.

### *Chem. Abstr. Name: Tetrachloromethane*

*Exposure to carbon tetrachloride may occur in its production, in the production of refrigerants, in laboratories and during degreasing operations. It has been detected at low levels in ambient air and water.*

### *5.2 Human carcinogenicity data*

*The risk of cancer from carbon tetrachloride has been examined in five occupational populations. In three of four studies that collected information on non-Hodgkin lymphoma (two cohort investigations and one independent nested case-control study), associations with exposure to carbon tetrachloride were suggested. However, not all of these studies distinguished exposure to carbon tetrachloride specifically, and the associations were not strong statistically. In the fourth study (another cohort investigation), few men were exposed to carbon tetrachloride and the risk of non-Hodgkin lymphoma was not reported. A nested case-control study of lung cancer in a cohort of chemical workers showed no association with exposure to carbon tetrachloride.*

*Four population-based case-control studies have examined associations of carbon tetrachloride with chronic lymphocytic leukaemia, brain cancer, female breast cancer and intraocular melanoma. Findings were generally unremarkable. In a fifth case-control study, which examined several cancers, no association was found with non-Hodgkin lymphoma, although the power to detect an increased risk was low.*

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<sup>17</sup> International Agency for Research on Cancer (1999). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 71: Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. Lyon, France.

### 5.3 Animal carcinogenicity data

*Carbon tetrachloride was tested for carcinogenicity by various routes of administration. It produced liver neoplasms in mice and rats and mammary neoplasms in rats following subcutaneous injection. In one study in mice by inhalation, an increased incidence of pheochromocytomas was reported. In experiments involving administration of carbon tetrachloride after known carcinogens, the occurrence of tumours and/or preneoplastic lesions of the liver in mice, rats and hamsters was enhanced.*

### 5.4 Other relevant data

*Carbon tetrachloride is metabolized by CYP2 enzymes; several reactive metabolites have been postulated, including radicals and phosgene. In vitro, DNA binding of carbon tetrachloride is observed in several cellular systems; no such binding in vivo has been reported.*

*Carbon tetrachloride induces hepatic cell proliferation and DNA synthesis. Carbon tetrachloride has a mutagenic effect and induces aneuploidy in several in-vitro systems.*

### 5.5 Evaluation

*There is inadequate evidence in humans for the carcinogenicity of carbon tetrachloride.*

*There is sufficient evidence in experimental animals for the carcinogenicity of carbon tetrachloride.*

#### *Overall evaluation*

*Carbon tetrachloride is possibly carcinogenic to humans (Group 2B).*

The International Agency for Research on Cancer (1995) concluded that tetrachloroethylene was probably carcinogenic to humans (Group 2A) based on limited evidence in humans and sufficient evidence in experimental animals of carcinogenicity.<sup>18</sup> The summary evaluation is reproduced below.

*Tetrachloroethylene is one of the most important chlorinated solvents worldwide and has been produced commercially since the early 1900s. Most of the tetrachloroethylene produced is used for dry cleaning garments; smaller amounts are used in the production of chlorofluorocarbons and for degreasing metals. About 513 thousand tonnes were used in all applications in western Europe, Japan and the United States in 1990.*

*Tetrachloroethylene has been detected in air, water, food and animal and human tissues. The greatest exposure occurs via inhalation, and workers in dry cleaning*

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<sup>18</sup> International Agency for Research on Cancer (1995). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 63: Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals. Lyon, France.

*and degreasing are the most heavily exposed. Individuals living or working in the vicinity of such operations have been shown to be exposed to lower concentrations.*

## *5.2 Human carcinogenicity data*

*Results relevant to assessing the relationship between exposure to tetrachloroethylene and cancer risk are available from five cohort studies. In one study in Finland and one in four states of the United States, exposure was specifically to tetrachloroethylene; biological monitoring was conducted in the Finnish study. In a cohort study in Missouri, United States, in which follow-up was from 1948 to 1978, tetrachloroethylene was the chemical to which predominant exposure had occurred since about 1960. Data for a few cancer sites were reported in two other cohort studies, one in Louisiana and one in Utah, United States, in which exposure was to both tetrachloroethylene and other chemicals. Although data on different levels or duration of exposure were available in some of the cohort studies, the number of observed cases in each category was generally too small to allow adequate statistical power for testing for a dose-response relationship. Data from six relevant case-control studies have also been reported. In the two cohort studies in which results for oesophageal cancer were reported, namely the four-state United States and Missouri studies, the relative risks were 2.6 and 2.1. Lack of data on smoking or alcohol consumption, both strong risk factors for this cancer, indicates caution in interpreting this observation. The relative risks for cervical cancer were increased in three cohort studies in which such results were reported; however, potential confounding factors associated with socioeconomic status could not be adjusted for. Elevated relative risks for non-Hodgkin's lymphoma were observed in all three cohort studies in which such results were reported. With respect to cancer of the kidney, no consistent pattern of elevated risk was seen in the three cohort studies in which such results were reported. Although a case-control study conducted in Montréal, Canada, showed an odds ratio of 3.4, this was not statistically significant, and the exposure in question was to degreasing solvents and not specifically to tetrachloroethylene. In the cohort study in Missouri, the relative risk for urinary bladder cancer was elevated but not statistically significant; little or no information was available from other studies. Five studies of people exposed to drinking-water contaminated with tetrachloroethylene have been reported. In four of these, no consistent pattern of risk for any specific cancers was observed. In the fifth study, in Massachusetts, United States, although the increase in the relative risk for leukaemia was significant, the result was based on only two cases. No consistent evidence for an elevated risk for leukaemia was seen in the cohort studies. In summary, there is evidence for consistently positive associations between exposure to tetrachloroethylene and the risks for oesophageal and cervical cancer and non-Hodgkin's lymphoma. These associations appear unlikely to be due to chance, although confounding cannot be excluded and the total numbers in the cohort studies combined are relatively small.*

## *5.3 Animal carcinogenicity data*

*Tetrachloroethylene was tested for carcinogenicity by oral administration in one experiment in mice, and a significant increase in the incidence of hepatocellular*

carcinomas was observed in animals of each sex. A study in rats treated orally was inadequate for an evaluation of carcinogenicity. Tetrachloroethylene was tested for carcinogenicity by inhalation in one experiment in mice and in one experiment in rats. The incidence of hepatocellular adenomas and carcinomas was significantly increased in mice of each sex, and the incidence of mononuclear-cell leukaemia was significantly increased in rats of each sex. A nonsignificant increase in the incidence of uncommonly occurring renal-cell adenomas and adenocarcinomas was also observed in male rats. Tetrachloroethylene did not induce skin tumours in mice after administration by topical application in one study.

A presumed metabolite of tetrachloroethylene, tetrachloroethylene oxide, did not increase the incidence of local tumours in mice when given by topical application or subcutaneous injection.

#### 5.4 Other relevant data

Tetrachloroethylene is rapidly absorbed after inhalation and from the gastrointestinal tract, but dermal absorption from the gaseous phase is negligible. The biotransformation of tetrachloroethylene is species- and dose-dependent; mice consistently had a greater capacity to biotransform tetrachloroethylene than rats. Two metabolic pathways have been demonstrated in rodents: cytochrome P450-catalysed oxidation and, as a minor route, glutathione conjugation.

Tetrachloroethylene shows only low acute toxicity in humans and in experimental animals. After repeated administration, the major target organ is the liver in mice and the kidney in rats. Tetrachloroethylene induced peroxisome proliferation in mouse liver after oral administration; a marginal response was observed in mouse kidney and rat liver.

Disturbances of sperm quality and fertility have been observed among dry cleaners exposed to tetrachloroethylene in a few studies of limited size. The results of studies of women exposed to tetrachloroethylene in dry cleaning shops and other settings are generally consistent in showing an increase in the rate of spontaneous abortions; however, other solvents were also present in most of these workplaces. Effects on other reproductive outcomes such as stillbirths, congenital malformations and low birth weight could not be evaluated in these studies.

Tetrachloroethylene can cross the placenta of rats and is metabolized in the placenta or fetus to trichloroacetic acid. Tetrachloroethylene appears to have little toxicity in developing rats and rabbits; high atmospheric concentrations produced delayed fetal development in mice in one study.

The frequencies of gene conversion and gene mutation were not increased in yeast recovered from mice treated with tetrachloroethylene in vivo. Tetrachloroethylene increased the frequency of DNA single-strand breakage/alkaline-labile sites in the liver and kidney of mice in vivo in one study, but binding to DNA was not demonstrated in mouse liver.

It did not induce gene mutation (in a single study), chromosomal aberrations, sister chromatid exchange (in a single study) or DNA damage in mammalian cells in vitro. In single studies, it induced morphological transformation in virus-infected rat embryo cells but not in BALBc/3T3 cells. The only study available showed no induction of gene mutation by tetrachloroethylene in insects.

Tetrachloroethylene did not usually induce gene conversion in yeasts; the results with regard to induction of aneuploidy in one study were inconclusive.

*Tetrachloroethylene did not increase the frequency of mutations in bacteria, except in one study in which a metabolic activation system consisting of liver and kidney fractions, which favours glutathione conjugation and further activation, was used. The metabolites formed from tetrachloroethylene in rats by minor biotransformation pathways, S-1,2,2-trichlorogluthathione and derived sulfur conjugates, were genotoxic in bacteria and cultured renal cells. The frequency of H-ras mutations was lower in hepatocellular tumours from tetrachloroethylene-treated mice than in tumours from control animals, whereas the frequency in hepatocellular tumours from trichloroethylene-treated mice was not significantly different from that in controls. The frequency of K-ras mutations was higher in liver tumours from tetrachloroethylene-treated mice than in tumours from control animals.*

### 5.5 Evaluation

*There is limited evidence in humans for the carcinogenicity of tetrachloroethylene. There is sufficient evidence in experimental animals for the carcinogenicity of tetrachloroethylene.*

#### Overall evaluation

*Tetrachloroethylene is probably carcinogenic to humans (Group 2A). In making the overall evaluation, the Working Group considered the following evidence:*

- (i) Although tetrachloroethylene is known to induce peroxisome proliferation in mouse liver, a poor quantitative correlation was seen between peroxisome proliferation and tumour formation in the liver after administration of tetrachloroethylene by inhalation. The spectrum of mutations in proto-oncogenes in liver tumours from mice treated with tetrachloroethylene is different from that in liver tumours from mice treated with trichloroethylene.*
- (ii) The compound induced leukaemia in rats.*
- (iii) Several epidemiological studies showed elevated risks for oesophageal cancer, non-Hodgkin's lymphoma and cervical cancer.*

IARC (1999) considered that hexachlorobutadiene was not classifiable as to its carcinogenicity to humans (Group 3), based on inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity of hexachlorobutadiene.<sup>19</sup>

Cullumbine (1957) [Chemical Defence Experimental Establishment, Porton, UK] reviewed the toxicity of screening smokes.<sup>20</sup> Chemical analysis of a hexachlorethane (HCE) smoke cloud produced in an enclosed gas chamber has shown that the constituent of the smoke likely to be harmful was zinc chloride. When inhaled, zinc chloride behaved as a corrosive irritant, and animal experiments had shown that it

<sup>19</sup> International Agency for Research on Cancer (1999). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 73: Some Chemicals That Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances. Lyon, France.

<sup>20</sup> Cullumbine H (1957). The toxicity of screening smokes. Journal of Royal Army Medical Corps 103: 119-122. ID 27841.

produced a severe tracheobronchitis and intense pulmonary congestion and oedema. Guinea-pigs, which were susceptible to bronchospasm, may die rapidly from this and at autopsy the lungs were markedly emphysematous. The dosage of zinc chloride required to produce death in animals was fairly large. The highest safe dosage had been taken as 2,000 mg per minute per cubic metre. This dosage was applicable for a single exposure or a series of repeated minor exposures over a period of ten days. The author discussed what this safety dose meant in terms of safe distance from the smoke source. It had been calculated that, in good area smoke screen at night, under suitable meteorological conditions, the concentration of zinc chloride at 200 yards from the source was about 85 mg per cubic metre and at 1,000 yards, it was about 13 mg per cubic metre. In efficient flank screening by day, the zinc chloride concentration at 100 yards from the source was about 47 mg per cubic metre and at 1,000 yards was about 0.9 mg per cubic metre. In a concentration of 120 mg per cubic metre of zinc chloride from HCE-mixture, volunteers complained of irritation of the nose, throat and chest, with cough and nausea, after two minutes. At 80 mg per cubic metre, the majority had slight nausea, and one or two coughed. The lethal dosage for humans was not known, but on the basis of animal experiments, it was considered to be probably greater than 50,000 mg per minute per cubic metre of zinc chloride. That dosage would be achieved by one generator in a 100 cubic foot room, in two to three minutes. It was usually following exposure in small, enclosed spaces, that the majority of reported casualties and deaths from HCE smoke had occurred (eg trenches, dug-outs, tunnels, between decks in ships). Affected men usually complained of chest and abdominal pain, difficulty in breathing, continuous coughing, nausea, and vomiting. Lacrimation and conjunctivitis may be seen. Dyspnoea and cyanosis become progressively more marked. In exceedingly high concentrations, such as have resulted from the spontaneous ignition of smoke generators in a tunnel, death may occur rapidly from asphyxia due to laryngeal oedema and spasm of the glottis. In lesser but still very high concentrations, death may occur in a few hours from severe haemorrhagic ulceration and excoriation of the upper respiratory tract. With smaller but still high dosage, the cause of death was usually pulmonary oedema and occurred within 24 to 48 hours. Those dying later, showed at autopsy, damage to the bronchial mucosa, moderate pulmonary oedema and a secondary bronchopneumonia.

Hjorsto et al (1988) discussed zinc chloride smoke.<sup>21</sup> Fumes from smoke ammunition bombs (hexite) consisted mainly of zinc chloride ( $\text{ZnCl}_2$ ) with an average particle size of 0.1 micron. 20% of the particles are deposited beyond the respiratory bronchioles, the remainder in the tracheobronchial tree. Zinc chloride is a corrosive heavy metal salt that immediately produces coughing and dyspnoea and can result in acute chemical pneumonitis. Several reports described fatal adult respiratory distress syndrome (ARDS) immediately following the inhalation of zinc chloride smoke. The early phase of lung injury is marked by increased permeability to plasma proteins and fluids through the alveolocapillary membrane. This led to interstitial and alveolar oedema. Later fibroblasts proliferate and collagen increases in the alveoli and interstitium. The largest incident occurred on Malta during World War II, when a smoke ammunition depot ignited in a tunnel injuring 70 people, 35 were hospitalised and 10 people died of ARDS within four days of exposure [Evans 1945]. Non-fatal zinc chloride smoke inhalation incidents have also been described. Coughing and dyspnoea occur immediately after zinc chloride inhalation. This is followed hours

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<sup>21</sup> Hjorsto E, Qvist J, Bud M, et al (1988). ARDS after accidental inhalation of zinc chloride smoke. *Intensive Care Med* 14: 17-24. ID 26870.



later by an elevation in core temperature and by diffuse consolidation on chest x-ray. Chest x-ray densities can remain after the patient has become asymptomatic. Nine Danish soldiers were hospitalised with upper airway irritation after a military training accident [Pedersen et al 1984]. None of the patients developed progressive lung disease and all were discharged after a few days. Johnson et al (1961) reported three patients with mild respiratory irritation. Vital capacity was reduced to 65-82% of the predicted value. The vital capacity returned to normal during convalescence. Schmahl (1974) reported three patients with respiratory complaints. In two, there was complete recovery of lung function, studied four weeks after inhalation. The third patient developed a spontaneous pneumothorax on day four after zinc chloride exposure and vital capacity did not return to normal until several months later. Reports in the literature of zinc chloride smoke exposure were summarised in the following table.

Study	Number of patients hospitalised	Number of patients died and days to death
Evans 1945	35 [70 exposed]	10 0-4 days
Pare et al 1954	1	0
Johnson et al 1961	3	0
Milliken et al 1963	2	1 18 days
Macaulay et al 1966	1	1 11 days
Schmahl 1974	11	0
Schiadt et al 1979	1	1 11 days
Schenker et al 1981	0 (81 exposed)	0
Pedersen et al 1984	9	0
Matarese et al 1986	1	0

Schaeffer et al (1988) analysed the chemical characteristics of residues from military HC smoke-pots.<sup>22</sup> The authors noted that deposited residues contained concentrations of Al, zinc and chlorinated hydrocarbons of more than 1000 mg/kg of residue and concentrations of cadmium, arsenic, lead and iron of about 500 mg/kg. The major organic compounds were HCE (100 to 500 ppm), hexachlorobenzene (5 to 150 ppm), hexachlorobutadiene (5 ppm) and phenols.

Cassel et al (1993) analysed smoke generated from zinc/hexachloroethane and titanium dioxide/hexachloroethane pyrotechnical mixtures in a closed space.<sup>23</sup> Several chlorinated organic substances were formed from both types of smoke munitions during combustion. The authors noted that some of these substances had well documented toxic and mutagenic effects, for example, hexachlorobenzene, hexachlorobutadiene and chlorinated dibenzofurans and dibenzodioxins. The amount

<sup>22</sup> Schaeffer D, Kapila S, Meadows J, et al (1988). Chemical characterization of residues from military HC smokepots. (Abstract). Journal of Hazardous Materials 17: 315-328.

<sup>23</sup> Cassel G, Fredriksson S, Faengmark I (1992). Zinc- and Titanium smoke: chemical composition and physical characteristics. (Abstract) Text Swedish. NTIS PB93-123487.

of by-products formed was higher from the titanium dioxide/hexachloroethane munition than from the zinc munition.

Brooks (1998) in his review of occupational asthma noted that there had been published reports of reactive airways disease syndrome (RADS) in relation to zinc chloride.<sup>24</sup>

Zerahn et al (1999) discussed the literature on pulmonary damage from zinc chloride smoke.<sup>25</sup> The author noted that in more severe cases emphysematous bullae and pneumothorax occurred. Exposure to high concentrations resulted in adult respiratory distress syndrome although the exact mechanism was unknown. The author identified three case reports where the gas transfer for carbon monoxide after exposure to zinc chloride smoke had been reported. It was reported as normal in one case (Allen et al 1992) and a slight reduction in DLCO four months after exposure was present in another case (Freitag and Caduff 1996 - German). The third case showed a moderate to severe reduction two weeks after exposure with recovery several months later (Wang et al 1987). Pathological findings in fatal human cases included extensive interstitial and intra-alveolar fibrosis, diffuse microvascular obliteration and widespread occlusion of the pulmonary arteries.

Greenfield et al (2002) discussed microbiological, biological, and chemical weapons of warfare and terrorism.<sup>26</sup> It was noted that zinc chloride was the principal component generated from "smoke bombs". Zinc chloride was an upper airway irritant and higher exposures resulted in lung injury and acute respiratory distress syndrome.

Zinc oxide/hexachloroethane smoke was recently reviewed (2002).<sup>27</sup> This article noted that high concentrations of this smoke in closed spaces had caused fatalities and HC munitions should not be used indoors or in closed compartments. Some countries required the use of a respirator whenever HC smokes were used. The HC smoke in common use contained zinc oxide and hexachloroethane. Upon burning, several compounds were produced: zinc chloride, zinc oxychloride, phosgene in low concentration, hydrogen chloride, tetrachloroethylene, carbon tetrachloride, and carbon monoxide. Many of these compounds had been implicated in the toxic reactions seen following exposure to HC smoke, but experimental studies clearly indicated that zinc chloride and zinc oxychloride inhalation produced a pattern of pulmonary injury identical to that seen following HC smoke inhalation. Acute effects of HC smoke exposure were said to include chemical pneumonitis and pulmonary oedema. Chronic effects following inhalation of HC smoke included focal atelectasis, bronchiolar-alveolar hyperplasia, and pulmonary fibrosis. The prognosis related to the extent of the pulmonary damage. At moderate exposures, some symptoms may persist for one to two weeks. In severe exposures, a permanent reduction in lung function may occur from pulmonary fibrosis. In severe exposures, pulmonary oedema may

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<sup>24</sup> Brooks S (1998). Occupational and environmental asthma. In: Environmental and Occupational Medicine Third Ed. W. Rom (Ed). Chapter 33, p 490-91. Lippincott-Raven ID B21

<sup>25</sup> Zerahn B, Kofoed-Enevoldsen A, Jensen B, et al (1999). Pulmonary damage after modest exposure to zinc chloride smoke. Respiratory Medicine 93: 885-90. ID 27446

<sup>26</sup> Greenfield R, Brown B, Hutchins J, et al (2002). Microbiological, biological, and chemical weapons of warfare and terrorism. Am J Med Sci 323: 326-340. ID 26445.

<sup>27</sup> Anon (2002). Smokes, fuels, and incendiary materials. J R Army Med Corps 148: 395-7. ID 28186.

result in possible death. The authors noted that more recently, concerns had been expressed about possible mutagenicity or carcinogenicity of some of the components of HC smoke, in particular unburnt hexachloroethane and tetrachloroethylene. However, in-vitro and in-vivo studies of unscheduled DNA synthesis had confirmed an overall lack of genotoxic effects in smoke condensates (Anderson et al 1996).

### **Animal Studies**

Clode et al (1991) studied the mutagenicity of a zinc oxide-hexachloroethane smoke in-vitro and in-vivo.<sup>28</sup> A Zn-HCE smoke was generated inside a specifically designed apparatus. Air containing 0 to 2800 micrograms per litre Zn/HCE was tested for mutagenicity in the Ames/Salmonella assay using strains TA-1535, TA-1537, TA-98, and TA-100, with or without metabolic activation from S9 mix. The bacteria were exposed to the smoke for one hour. Zn/HCE smoke showed weak mutagenicity in strains TA-1535 and TA-1537 in the absence of S9 mix and in TA-100 in the presence and absence of S9 mix. CD-1-mice were exposed for one hour to 0, 22, 77 or 117 ug/L Zn/HCE smoke. They were killed 24, 48 or 72 hours later and femoral bone marrow tested. The smoke did not significantly affect the frequency of micronucleated polychromatic erythrocytes or normochromatic erythrocyte micronuclei. The authors concluded that Zn/HCE smoke was weakly mutagenic in the Ames/Salmonella assay but did not cause chromosome damage in mouse bone marrow.

Anderson et al (1996) used an in-vitro and in-vivo unscheduled DNA synthesis (UDS) assay in rat hepatocytes to study the effects of a zinc oxide/hexachloroethane (Zn/HCE) smoke.<sup>29</sup> In the in-vitro investigation, at the highest concentration of Zn/HCE smoke, where some viable cells were seen, an increase in UDS was observed. However, this increase was not statistically significant, was only seen at a level where toxicity was observed and was therefore not considered to be biologically significant. In the in-vivo investigation, two doses of Zn/HCE smoke were used (Zn content of 20 and 56 ug/l air). A dose-related increase in UDS was observed which was not statistically significant. The positive control behaved as anticipated, showing a highly statistically significant response. The authors concluded that Zn/HCE smoke did not induce unscheduled DNA repair in the in-vitro or in-vivo UDS assays.

Rodilla et al (1998) evaluated the toxicity of several inorganic metal compounds, including zinc chloride, and the induction of metallothionin by these compounds in cultured human renal proximal tubular cells for up to four days.<sup>30</sup> Metallothionin (MT) is a metal-binding protein within the cell, has been shown to be induced by toxic metals and may play a role in cell protection. Cell toxicity was seen only after the highest dose of zinc chloride was used, 100 uM ( $p < 0.01$ ). Zinc chloride also induced metallothionin as revealed by immunocytochemistry. Maximal induction occurred two days after treatment. However, there was no correlation between metal

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<sup>28</sup> Clode S, Riley R, Blowers S, et al (1991). Studies on the mutagenicity of a zinc oxide-hexachloroethane smoke. (Abstract). Human and Experimental Toxicology 10: 49-57.

<sup>29</sup> Anderson D, Blowers S, Marrs T, et al (1996). An in vitro and an in vivo unscheduled DNA synthesis assay with a zinc oxide/hexachloroethane (ZN/HCE) smoke. (Abstract) Hum Exp Toxicol 15: 38-44.

<sup>30</sup> Rodilla V, Miles A, Jenner W, et al (1998). Exposure of cultured human proximal tubular cells to cadmium, mercury, zinc and bismuth: toxicity and metallothionin induction. Chemico-Biological Interactions 115: 71-83. ID 27429.

induced MT and cell susceptibility to a particular metal. A significant degree of variation between the different cell culture isolates was observed. The renal cells cultured also came from nephrectomy specimens of patients with renal cancer. The authors noted that zinc had a relatively low toxicity in-vivo (except when exposure was by inhalation), probably due to its rapid redistribution within the body. Evidence of renal toxicity had not been observed in individuals ingesting as much as 12 g of elemental zinc over a two-day period. Nor was it seen in experimental animals exposed to zinc doses 100 times the dietary requirement. Other in-vitro studies in cultured cells of human or rodent origin had produced mixed results; some had shown zinc at high concentrations to be cytotoxic but others had not. However, the authors also noted that the difference between the low toxicity of zinc observed in-vivo and some cytotoxicity seen in in-vitro studies could be explained by the absence of two important regulatory mechanisms in in-vitro systems. Namely, that zinc distribution in-vivo was regulated by homeostatic mechanisms that acted mainly on absorption and liver levels of the metal and the level of zinc excretion in the urine can be relatively high.

Yurkow and DeCoste (1999) conducted an in-vitro laboratory study on the effects of cadmium chloride and zinc chloride on metallothionein (MT) levels in human peripheral blood leukocytes.<sup>31</sup> Flow cytometry was used to characterise the basal and metal-induced expression of MT in monocytes, lymphocytes and granulocytes of healthy male donors (aged 28-39 years, nonsmoking, nonatopic based on circulating immunoglobulin E levels). The exact number of donors was not specified. Cells were cultured for 24 hours. Both cadmium chloride (3  $\mu$ M) and zinc chloride (50  $\mu$ M) induced MT expression in monocytes to a similar degree and did not affect the expression of this protein in granulocytes, cadmium but not zinc treatment induced dramatic increases in MT levels of lymphocytes. The authors noted that in contrast to their results, other investigators have shown zinc-induced MT expression in cultures of human lymphocytes (Mesna et al 1990, Yamada and Koizumi 1991). However, most of these studies used high levels of zinc (ie 200  $\mu$ M) and / or prolonged induction periods (ie 6 days). The authors considered that this meant that zinc was a relatively poor inducer of MT in the human lymphocyte.

Wilhelm et al (2001) conducted an in-vitro laboratory study on the effects of zinc chloride on cellular glutathione in cultured human and rat lung cell lines.<sup>32</sup> The cell lines were 16Lu [fibroblast-like human lung], A549 [malignant alveolar-epithelial human lung] and L2 [alveolar-epithelial like rat lung]. Cell lines were incubated for up to 36 hours. Inhibition of the enzyme glutathione reductase was the initial cellular effect; it occurred in the different cell lines after about 0.5 hour of zinc chloride exposure. Depletion of glutathione was observed in the human non-malignant and malignant lung cell lines after 0.5 and 8 hours of zinc chloride exposure respectively and in the rat lung cell line after about one hour. An increase in oxidised glutathione was observed in human non-malignant and malignant lung cell lines after 3 and 12 hours of zinc chloride exposure respectively and in the rat lung cell line after three hours. A decrease in glutathione synthesis was found in all three cell lines after

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<sup>31</sup> Yurkow E, DeCoste C (1999). Effects of cadmium on metallothionein levels in human peripheral blood leukocytes: a comparison with zinc. *Journal of Toxicology and Environmental Health Part A*, 58: 313-27. ID 27744

<sup>32</sup> Wilhelm B, Walther U, Fichtl B (2001). Effects of zinc chloride on glutathione and glutathione synthesis rates in various lung cell lines. *Arch Toxicol* 75: 388-94. ID 27427

exposure to zinc chloride; after one hour in human non-malignant lung cells, after two hours in rat lung cells and after eight hours in human malignant lung cells. The authors suggested that the toxic effects of zinc may be mediated via glutathione.

Santra et al (2000) studied chromosome damage induced by three zinc compounds (zinc chloride, zinc sulfate, zinc acetate) in human leukocytes in-vitro.<sup>33</sup> Blood samples from six healthy donors were used for each zinc salt. Three concentrations of each salt were added to leukocyte cultures (0.00003 M, 0.0003 M, 0.0015 M). The cells were harvested after 48 and 72 hours. Control cultures where water or DMSO was added were also kept. The endpoints screened were chromosomal aberrations per cell and the percentage of damaged cells. 100 metaphases were scored. Chromosomal aberrations included chromatid breaks, chromosome breaks, and rearrangements but gaps were not included. All three zinc salts were lethal at the highest concentration. The frequency of chromosomal aberrations per cell was significantly increased for the two lower doses for all three zinc salts compared to controls. For an inoculation time of 24 hours and a harvest time of 72 hours, the frequency of chromosomal aberrations per cell for both zinc chloride doses was 0.04 versus 0.005 for the control culture ( $p < 0.001$ ). The increase in frequency of chromosomal aberrations per cell was not related to the concentration of zinc chloride used. No results for the endpoint, percentage of damaged cells, were provided.

Several articles also discussed animal studies.<sup>34 35</sup> Animal studies have also demonstrated pulmonary damage following exposure to smoke from hexachloroethane-zinc oxide mixtures. Karlsson et al (1986) observed hyperaemia, parenchymal haemorrhage, oedema, bronchial dilatation and bullous emphysema in rat lungs after low and moderate nonfatal exposure. Marrs et al (1983) studied rats and rabbits and demonstrated early death after moderate exposure as a result of tracheal inflammation and necrosis and delayed death as a result of pulmonary oedema and bronchopneumonia. Extensive pulmonary fibrosis in rats was demonstrated (Brown et al 1990). Repeated exposure in mice was associated with pulmonary oedema, alveolar haemorrhage, localised bullous emphysema and alveolar cell carcinoma (Marrs et al 1988). A 15% decline in haemoglobin was seen in rats after prolonged ingestion of food containing zinc chloride (Zaporowska et al 1992). Spermatic chromatin structure was altered in male rats fed diets that contained zinc chloride (Evenson et al 1993).

### **Case Reports / Series**

Evans (1945) described a case series of individuals exposed to high concentration of zinc chloride smoke in an enclosed space in Malta in 1943.<sup>36</sup> Smoke generators that contained hexachloroethane and zinc oxide, together with small quantities of calcium silicide and an igniter compound exploded in a tunnel 200 hundred yards long. The resulting smoke consisted mainly of zinc chloride, with some carbon and carbon

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<sup>33</sup> Santra M, Talukder G, Sharma A (2000). Comparison of chromosome damage induced by three zinc compounds using human leukocyte culture. *Biological Trace Element Research* 78: 113-119. ID 27783

<sup>34</sup> Allen M, Crisp A, Snook N, et al (1992). Smoke-bomb pneumonitis. *Respiratory Medicine* 86: 165-166. ID 27448

<sup>35</sup> Holmes P (1999). Pneumomediastinum associated with inhalation of white smoke. *Military Medicine* 164: 751-2. ID 27447

<sup>36</sup> Evans E (1945). Casualties following exposure to zinc chloride smoke. *Lancet* 2: 368-370. ID 27449

dioxide. 70 individuals inside the tunnel at the time were exposed to the zinc chloride smoke. 34 required treatment at a first aid station and eleven of these were hospitalised. Another 10 individuals died; three within minutes, two within hours, two on day three and two on day four. Deaths that occurred immediately or within hours were considered a result of pulmonary oedema and shock. Deaths that occurred later resulted from bronchopneumonia (as a complication of the initial lesion). The clinical picture was of acute inflammation of the entire respiratory tract; nasopharynx, larynx, trachea, bronchi and lungs. Symptoms included pain in the throat and nasopharynx, hoarse voice, laryngeal stridor, dyspnoea, chest constriction, retrosternal pain, cyanosis, and cough productive of sputum (sometimes bloody). Other symptoms included headache, epigastric pain, nausea and vomiting. Six weeks after exposure, most of those that survived were no longer confined to bed. One patient developed a lung abscess and another patient was still convalescing and these two were still bed-bound. The long-term respiratory sequelae were not stated. An autopsy on two of the fatal cases was performed. The first case had died within four hours of the exposure and autopsy revealed red and very oedematous larynx, trachea, bronchi and lungs with right-sided cardiac dilatation. The second case had died on the third day and autopsy showed similar but more advanced change in the respiratory tract as well as bronchopneumonia, and a reddened mucus membrane of the stomach and duodenum. Interestingly, in the non-pneumonia areas of the lungs, there were areas of oedema, haemorrhage and acute emphysema. All hospitalised subjects also had reddened conjunctivae, which responded well to treatment. However, two patients also had corneal ulceration, which required their transfer to an ophthalmologist after the first week. Their long-term ocular outcome was not reported. Four hospitalised patients also had superficial burns to the face and hands that also responded well to treatment. Evans also described another incident in Malta when zinc chloride smoke was used to screen the harbour and people were exposed in an open environment. A few people developed minor symptoms of slight respiratory distress, a feeling of chest constriction, dry throat and slight cough that lasted a few hours.

Lumsden and Weir (1945) described the case of a 28-year old Arab male with subglottic stenosis after exposure to an extremely dense cloud of screening smoke (zinc chloride) that issued from a room below, where smoke generators had exploded.<sup>37</sup> The patient was hospitalised two hours after exposure with stridor, harsh cough with frothy sputum, vomiting and a few rhonchi. Fourteen hours later, the patient complained of a sore throat, retrosternal pain and continuing productive cough. Temperature and respiratory rate were elevated. Blisters developed on the ears and hands [these subsequently healed]. The bronchitis resolved in five days. Although the patient complained of sore throat and hoarseness. Indirect laryngoscopy two weeks after exposure revealed a yellowish-white membrane over the anterior surface of the larynx, that involved both ventricular bands and vocal cords, as well as generalised oedema and redness. Three weeks after exposure, stridor developed which became recurrent and required tracheostomy four weeks after exposure. Six weeks after exposure, laryngoscopy showed marked oedema of the arytenoids and ventricular folds with stricture of the glottic aperture. About three and half months after exposure, these laryngeal changes were still present. During the next six weeks, the patient had two attacks of bronchitis with pneumonitis. Seven and half months after exposure, direct laryngoscopy showed marked subglottic narrowing with

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<sup>37</sup> Lumsden R, Weir C (1945). Subglottic stenosis after exposure to a high concentration of screening smoke (zinc chloride). *BMJ* April 21: 554-5. ID 27808

granulations blocking the lumen. Histology was reported as oedematous granulation tissue with fibrosis in the deeper layers. The appearances were consistent with some mechanical or chemical factor but not infection. 13 and a half months after exposure and four months after continuous dilatation treatment, the tracheostomy tube was removed. The patient required subsequent periodic dilatation.

Whitaker (1945) described the case of a 20-year old male sailor who developed pulmonary oedema / pneumonitis following exposure to a smoke screen containing zinc chloride.<sup>38</sup> The patient developed acute respiratory distress, dyspnoea and cyanosis immediately after exposure. A chest x-ray about two weeks after exposure showed shadowing around both lung roots and discrete mottling throughout both lung fields. About three weeks after exposure there was marked clinical improvement but the chest x-ray was still abnormal. About 10 weeks after exposure, the patient had returned to normal health and his chest x-ray was clear apart from some fibrosis of the lung roots (stated to be within normal limits). Secondary infection was not apparent.

Pare and Sandler (1954) described the case of a previously healthy 18-year old soldier hospitalised with pneumonitis following exposure to a smoke bomb.<sup>39</sup> Exposure to the smoke, (thought to be zinc chloride smoke from a hexachloroethane-zinc oxide smoke bomb), occurred inside a house and lasted about ten minutes. The subject developed coughing, dyspnoea, vomited and felt sleepy. One day later he had epistaxis and a raised temperature. Moist crepitations were audible in the lungs, five to six days after exposure. A chest x-ray showed patchy consolidation of both lungs. About one month after exposure, the subject had improved clinically but the chest x-ray was not considered normal until about seven weeks after exposure. The subject was clinically and radiologically normal at follow-up, 10 months after exposure.

Johnson and Stonehill (1961) described three cases of chemical pneumonitis in young airmen with excessive exposure to smoke that contained zinc chloride.<sup>40</sup> They were treated at a US Air Force hospital. Initial symptoms included burning of the throat, paroxysmal cough, dyspnoea, tightness of chest and nausea and these disappeared after about six hours of conservative care. Fever, tachypnoea and cyanosis developed later. Coughing was non-productive and infrequent despite the presence of marked parenchymal infiltration on chest x-ray. Auscultation of the lungs revealed only occasional wheezes and rales in one of the patients. The vital capacity was reduced during the acute illness. 24-hour urinary zinc excretion was initially elevated in two of the patients. All three patients were essentially asymptomatic after three to four days but pulmonary infiltrates on chest x-ray did not clear for about one month to six weeks. No lasting effects were detected in follow-up symptoms, chest x-rays, respiratory function tests or blood gas studies. An analysis of the combustion products of a grenade containing zinc oxide, grained aluminum and hexachloroethane demonstrated mainly zinc chloride (4,075 mg per cubic metre).

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<sup>38</sup> Whitaker P (1945). Radiological appearances of the chest following partial asphyxiation by a smoke screen. *British Journal of Radiology* 18: 396-7. ID 27906

<sup>39</sup> Pare CMB, Sandler M (1954). Smoke bomb pneumonitis: description of a case. *J R Army Med Corps*, 100:320-323. ID 28384

<sup>40</sup> Johnson F, Stonehill R (1961). Chemical pneumonitis from inhalation of zinc chloride. *Dis Chest* 40: 619-624. ID 27638

Milliken et al (1963) presented the case of a previously healthy fireman who was exposed to zinc chloride smoke from a smoke bomb during a fire-fighting demonstration.<sup>41</sup> The smoke generator had been ignited at the bottom of a depression a few feet below ground level, surrounded by buildings on three sides. The subject was exposed to the smoke for several minutes. He presented to hospital a few minutes after exposure and complained of nausea, sore throat and chest tightness. Thirty hours after exposure the respiratory rate rose to 50 per minute, cyanosis, confusion and coma occurred. 18 days after exposure the patient died. The patient developed acute interstitial pulmonary oedema, advanced interstitial pulmonary fibrosis, acute cor pulmonale and right ventricular hypertrophy. Findings were confirmed by post-mortem examination.

Macaulay and Mant (1964) reported the case of a 19 year old male soldier who died following a four-minute exposure to zinc chloride smoke from a military smoke canister in a confined space during a civil defence exercise.<sup>42</sup> The subject developed retrosternal pain, abdominal cramps and cyanosis within two hours of exposure. Acute pulmonary oedema was evident on x-ray 24 hours after exposure. Bronchopneumonia developed on the ninth day and death occurred on the eleventh day after exposure. Autopsy examination revealed consolidation of the lungs with oedema, necrosis, haemorrhage, and infarct in the left upper lobe. Microscopic examination revealed severe interstitial and alveolar fibrosis, with necrosis and squamous metaplasia of the large bronchi and oedematous exudate. Numerous smoke particles were found.

Matarese and Matthews (1986) described the case of a 20-year old man exposed to zinc chloride smoke from a military smoke grenade inside a closed civilian vehicle for about five minutes.<sup>43</sup> Three hours after exposure he was seen at the local hospital with choking sensation in pharynx, pleuritic chest pain, dyspnoea, chest tightness and wheezes on examination. Two days later he was re-seen with worsening respiratory symptoms, fever, productive cough and chest x-ray showed bilateral diffuse infiltrates. The patient was diagnosed with an acute respiratory distress-like syndrome. Two weeks after exposure he was seen again with respiratory symptoms, bloody sputum and a chest x-ray showed left pneumothorax. A later chest x-ray following reexpansion of the lung revealed several emphysematous blebs in both lung fields. Chest x-ray five months after exposure showed resolution of some bullae but persistent change also remained.

Malo and Cartier (1987) described two cases of new-onset occupational asthma in galvanised metal solderers exposed to fumes containing zinc.<sup>44</sup> Two solderers exposed to fumes of galvanised metal reported a history of shortness of breath and fever that occurred during the evening and night of days spent at work. One subject had been exposed to the fumes of galvanised metal for about one and a half years before the onset of symptoms while the onset was after the second exposure in the

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<sup>41</sup> Milliken J, Waugh D, Kadish M (1963). Acute interstitial pulmonary fibrosis caused by a smoke bomb. *Canad Med Assoc J* 88: 36-39. ID 27781

<sup>42</sup> Macaulay M, Mant A (1964). Smoke-bomb poisoning. A fatal case following the inhalation of zinc chloride smoke. (Abstract). *Journal of the Royal Army Medical Corps* 110: 27-32.

<sup>43</sup> Matarese S, Matthews J (1986). Zinc chloride (smoke bomb) inhalational lung injury. *Chest* 89: 308-9. ID 27450

<sup>44</sup> Malo J-L, Cartier A (1987). Occupational asthma due to fumes of galvanised metal. *Chest* 92: 375-377. ID 4360



other subject. Subjects had evidence of an obstructive deficit on spirometry. Bronchial hyper-responsiveness to histamine was significant in one subject and borderline in the other subject. Specific inhalation challenges performed by subjects soldering on galvanised iron revealed a late bronchospastic reaction (drop in FEV1 maximal three hours in one subject and five to nine hours in the other subject after soldering had ended). Environmental measurements demonstrated the presence of zinc after soldering on galvanised metal. The authors considered that bronchoconstriction was due to sensitisation to zinc.

Wang et al (1987) described a case of a previously healthy non-smoking male soldier exposed to zinc oxide-hexachloroethane smoke bomb while taking part in army jungle training.<sup>45</sup> He activated a smoke bomb under his poncho and immediately developed a burning sensation in his throat and severe dyspnoea. The patient was seen at a Singapore hospital two weeks after exposure with slight cough and dyspnoea on exertion. X-rays showed diffuse fine nodules in both lung fields, pneumomediastinum and subcutaneous emphysema. Pulmonary function tests showed a restrictive pattern and carbon monoxide diffusing capacity was reduced to approximately half of the predicted value. The author considered these findings consistent with a diagnosis of pulmonary oedema. Three months after exposure the patient was asymptomatic and chest x-ray was normal. Nine months after exposure, pulmonary function tests had returned to normal. Two years after exposure, the patient was well with normal chest x-ray and respiratory function tests.

Hjortso et al (1988) presented a case series of five soldiers injured by inhalation of hexite smoke ( $\text{ZnCl}_2$ ) during military training.<sup>46</sup> During a 1985 training exercise, five soldiers passed through a zinc chloride smoke-filled pipeline (30 metres long, 1.75 metres internal diameter). Two of the soldiers removed their gas masks and the gas masks were ill fitting in the other three soldiers. The two soldiers not wearing gas masks breathed hexite for one to two minutes. These soldiers were previously healthy 20-year old males. Both of these soldiers immediately developed severe coughing and dyspnoea. Blood pressure, pulmonary auscultation, and chest x-rays were normal on hospital admission. However, both soldiers slowly developed severe adult respiratory distress syndrome over the ensuing two weeks. This slow progressive clinical course had not been previously described. In both patients, an increased plasma zinc concentration was measured three weeks after the incident. Both patients died of severe respiratory failure (25 and 32 days after inhalation). At autopsy diffuse microvascular obliteration, widespread occlusion of the pulmonary arteries and extensive interstitial and intra-alveolar fibrosis were observed on pathology. The other major organs showed acute congestion and the kidneys showed acute tubular necrosis in one patient on pathology. Post-mortem zinc concentration was elevated in striated muscle in both patients and in lung tissue in one patient. There was no evidence of secondary infection - frequent blood and sputum cultures from both patients were normal, autopsy culture of blood and lung did not grow bacteria or fungi. Three other soldiers wearing ill fitting gas masks, immediately developed severe coughing and dyspnoea. They were hospitalised for several days. Their clinical course improved. 12 months after exposure their lung function tests were nearly normal; FEV1 varied from

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<sup>45</sup> Wang Y-T, Lee L, Poh S-C (1987). Phosgene poisoning from a smoke grenade. *Eur J Respir Dis* 70: 126-128. ID 27853

<sup>46</sup> Hjortso E, Qvist J, Bud M, et al (1988). ARDS after accidental inhalation of zinc chloride smoke. *Intensive Care Med* 14: 17-24. ID 26870.

72% to 86% (normal 82%-118%) and VC varied from 89% to 91% (normal 85% to 115%). However, all still had dyspnoea on exercise. Homma et al (1992) reported the pathological findings on these two fatal cases in more detail.<sup>47</sup>

Weir et al (1989) described two cases of occupational asthma due to soft corrosive soldering fluxes containing zinc chloride and ammonium chloride.<sup>48</sup> The subjects were employed in metal jointing. The diagnosis was based on work related deterioration in daily peak expiratory flow rate. The bronchoprovocation test was positive and there were changes demonstrated in PC (20) [provocative concentration producing a 20% fall in FEV1]. Symptoms of chest tightness and wheeze began in both subjects 18 months and 12 months respectively after beginning at work. Neither had a pre-existing history of asthma. In the first subject, there was a 26% fall in FEV1 after 15 minutes exposure to the flux. There was a 10% fall in FEV1 at ten minutes and a later 16% fall 7.5 hours after challenge with ammonium chloride. No change in FEV1 was observed after exposure to zinc chloride alone. In the second subject there was a fall in FEV1 of 19% after 15 minutes exposure to the flux. Bronchial responsiveness to inhaled histamine increased after challenge to the flux, with a ten fold fall in PC20 from 0.5 ml/mg before the challenge to 0.05 mg/ml 24 hours after the challenge. Both subjects had raised IgE concentrations. Both fluxes contained ammonium chloride and zinc chloride. Zinc chloride was thought to be part of the sensitising agent in subject one, since the fall in FEV1 after ammonium chloride was not as great as after the flux.

Demeter and Cordasco (1990) presented 19 cases of reactive airways dysfunction syndrome.<sup>49</sup> Eleven of the cases were associated with a joint exposure to zinc chloride fumes inside an office building. It was thought that the fumes were generated when a hydrochloric acid solution spilt onto a generator. Although this was not verified by any measurement or analysis of the fumes. Subjects required emergency treatment for dyspnoea, dizziness, cough, nausea and initial hypoxaemia (the latter in several subjects with the greatest fume exposure). This consisted of intravenous steroids, inhaled bronchodilators and oxygen (the latter was required in seven patients). X-rays did not detect abnormalities consistent with adult respiratory distress syndrome. Symptoms of chronic asthma persisted following the fume exposure. Four had symptoms for at least six months, one for at least one year, one for at least 1.5 years, two for at least 2.5 years, one for at least 5.5 years and two for at least 6.5 years. In four subjects, symptoms were stable and in seven subjects symptoms regressed. Only one subject had a past history of asthma, which had not been active before the exposure. In three patients, the diagnosis was based on a positive methacholine challenge and a positive bronchodilator challenge. In a further four patients, the diagnosis was confirmed by a positive bronchodilator challenge and in the remaining four patients the diagnosis was made on clinical grounds including spirometry. Three patients were current smokers and three had been smokers in the past. Following the exposure, patients commonly had multi-system complaints during convalescence. Ten

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<sup>47</sup> Homma S, Jones R, Qvist J, et al (1992). Pulmonary vascular lesions in the adult respiratory distress syndrome caused by inhalation of zinc chloride smoke: a morphometric study. *Hum Pathol* 23: 45-50. ID 27807

<sup>48</sup> Weir D, Robertson A, Jones S, et al (1989). Occupational asthma due to soft corrosive soldering fluxes containing zinc chloride and ammonium chloride. *Thorax* 44: 220-223. ID 27811

<sup>49</sup> Demeter S, Cordasco E (1990). Reactive Airways Dysfunction Syndrome: A Subset of Occupational Asthma. *Journal of Disability* 1: 24-39. ID 028283

of the eleven subjects complained of sinus symptoms and many had gastrointestinal complaints. Four patients had endoscopically proven oesophagitis/gastritis and three of these required treatment for greater than two years.

Allen et al (1992) described the case of a 25-year old healthy, non-smoking miner and territorial army soldier exposed to zinc chloride smoke from a hexachloroethane-zinc oxide smoke bomb for about 10 minutes in an enclosed environment.<sup>50</sup> Six other soldiers had lower exposure. All seven soldiers developed eye irritation, cough, retrosternal tightness and vomiting. They were admitted to an army hospital, given intermittent oxygen therapy and discharged the following day. Six soldiers were reviewed 10 days after the exposure. Two had developed dry cough and mild dyspnoea within 48 hours. All had normal chest x-rays and spirometry. The more heavily exposed soldier was seen 60 hours after exposure with worsening respiratory distress, fever, bilateral basal crackles, widespread alveolar shadowing on chest x-ray, and restrictive defect on spirometry [FEV1 51% predicted and FVC 45% predicted]. A diagnosis of pneumonitis was made and treatment included inspired oxygen, intravenous steroids and penicillamine. There was gradual improvement over two weeks. Twelve months after exposure, the patient still had mild dyspnoea on exercise and subnormal respiratory function tests [FEV1 75%, FVC 79%, RV 51%].

Freitag and Caduff (1996) reported the case of a previously healthy 19-year old male exposed to concentrated hexite smoke ( $\text{ZnCl}_2$ ) for several minutes during military training.<sup>51</sup> The initial symptoms of vomiting, cough and dyspnoea disappeared after a few hours. After 48 hours, the subject required hospitalisation for acute respiratory distress syndrome. Spirometry at this time showed a restrictive deficit; vital capacity 50% of predicted. Four months after exposure, the subject was not symptomatic but there was still a slight reduction in carbon monoxide diffusion.

Holmes (1999) described the case of a 26-year old healthy soldier exposed to zinc chloride smoke from a hexachloroethane smoke canister while lying on the ground.<sup>52</sup> He required hospital treatment for uncontrollable paroxysmal cough, sore throat, chest tightness and painful inspiration of seven hours duration following exposure. Examination revealed subcutaneous emphysema and bilateral wheezes. X-rays confirmed subcutaneous emphysema and chest x-ray demonstrated a small pneumomediastinum without pneumothorax. Clinical improvement occurred over several days. The author mentioned a recent report of a United Nations soldier in Bosnia who died within hours of an enclosed exposure after a HC canister was discharged into a tent (not referenced).

Zerahn et al (1999) described 13 Danish soldiers exposed to zinc chloride smoke during a routine military exercise.<sup>53</sup> Two smoke canisters were ignited in open air but smoke drifted into a building where the exercise was being conducted. Two of the unprotected soldiers were exposed for five to ten minutes inside the building while the

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<sup>50</sup> Allen M, Crisp A, Snook N, et al (1992). Smoke-bomb pneumonitis. *Respiratory Medicine* 86: 165-6. ID 27448

<sup>51</sup> Freitag A, Caduff B (1996). ARDS due to military hexite smoke exposure. *Schweizerische Medizinische Wochenschrift (English Abstract)* 126: 1006-10.

<sup>52</sup> Holmes P (1999). Pneumomediastinum associated with inhalation of white smoke. *Military Medicine* 164: 751-2. ID 27447

<sup>53</sup> Zerahn B, Kofoed-Enevoldsen A, Jensen B, et al (1999). Pulmonary damage after modest exposure to zinc chloride smoke. *Respiratory Medicine* 93: 885-90. ID 27446

remaining soldiers had transient exposure. The soldiers were aged from 19 to 26 years and eleven were never smokers. The soldiers were hospitalised on the day of exposure with mild symptoms of throat soreness, coughing, chest pain and headache. Twelve soldiers were treated with steroids. One soldier had persistent coughing and chest pain and a laryngoscopy five days after exposure showed hypopharyngeal inflammation, diagnosed as a chemical pharyngitis. These soldiers were followed with serial lung function tests, up until 29 weeks after exposure. Four weeks after exposure, a significant decline from baseline values in lung diffusion capacity and total lung capacity of 16.2% and 4.3%, respectively, was observed. At 29 weeks after exposure, the lung diffusion capacity was slightly ( $< 5\%$ ) though still significantly decreased ( $p < 0.05$ ). Chest x-rays and arterial blood gas analyses remained normal during follow-up. Plasma fibrinogen level was significantly elevated between one to eight weeks after exposure (compared to baseline values) and some peak values exceeded the normal range. Plasma zinc level was elevated above baseline values at two to eight weeks after exposure but levels remained within the normal range. The authors considered that the changes were compatible with an ulcerative and fibrotic process affecting lung alveoli and smaller bronchioli and could not exclude the possibility of permanent damage.

Pettila et al (2000) described three cases of adult respiratory distress syndrome after exposure to zinc chloride smoke.<sup>54</sup> The first two patients were with four other men inside a small cottage when a military grey smoke bomb was discharged. The estimated time of exposure was one to five minutes. The first patient was a 37-year old man with a long history of alcohol abuse and smoking while the second patient was a 39-year old male. Both were admitted to hospital two to three days after exposure with dyspnoea. Respiratory failure developed on day five and chest x-rays showed diffuse pulmonary interstitial infiltrates. Both patients died despite treatment, patient two on day 12. Autopsy showed necrotic lungs in both patients and lobar pneumonia in the first patient. The third patient was a healthy 18-year old male exposed to grey smoke inhalation during military training. The grey smoke bomb contained 43% zinc chloride, 52% hexachloroethane and 5% trinitrotoluene. The estimated exposure time was two to five minutes. Respiratory distress developed on day two, and diffuse pulmonary infiltrates, subcutaneous emphysema and pneumomediastinum were seen on chest CT. Emphysematous bullae were detected and required videothoroscopic excision on day 57. Recurrent pneumothorax occurred and required chemical pleurodesis. The patient survived after prolonged intensive care but suffered permanent lung damage. Four months after smoke inhalation, pulmonary function tests showed an FEV1 44% of predicted and an FVC 41% of predicted which indicated a severe restrictive pulmonary dysfunction. The patient was able to walk only 400 m in six minutes.

## Surveys

Schenker et al (1981) surveyed participants at a US airport disaster drill exposed to zinc chloride smoke as a result of detonation of a smoke bomb that consisted of hexachloroethane, zinc oxide and calcium silicide.<sup>55</sup> Participants played the part of

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<sup>54</sup> Pettila V, Takkunen O, Tukianen P (2000). Zinc chloride smoke inhalation: a rare cause of severe acute respiratory distress syndrome. *Intensive Care Med* 26: 215-7. ID 27428.

<sup>55</sup> Schenker M, Speizer F, Taylor J (1981). Acute upper respiratory symptoms resulting from exposure to zinc chloride aerosol. *Environmental Research* 25: 317-324. ID 27473.

victims or were physician or nurse attendants. Exposure to zinc chloride smoke was in the open. 52% of victims (70 / 135) and 11 of at least 28 attendants completed a questionnaire one to two days after the exposure. 12 subjects had no, 16 subjects had little, 27 subjects moderate and 26 subjects heavy exposure to the zinc chloride smoke. Symptoms present at any time in the 48 hours following smoke exposure and the proportion of affected subjects with no and heavy exposure follow. Cough [8.3% of unexposed, 96.2% of heavy exposure]; light-headed [0%, 80.8%]; sore throat [16.7%, 76.9%]; listless [0%, 76.9%]; metallic taste [8.3%, 73.1%]; chest tightness [0%, 61.5%]; hoarseness [0%, 61.5%]; soreness in chest [0%, 57.7%]; nausea [0%, 53.8%]; drunk sensation [0%, 46.2%]; wheezing [0%, 42.3%]; and vomiting [0%, 7.7%]. Hence symptoms increased with increasing exposure. Eleven subjects reported onset of some symptoms that lasted at least six hours, from two to twenty hours after smoke exposure. These symptoms were mostly nausea, fatigue or headache. Seven subjects noted symptoms that persisted after 48 hours. Sore throat or hoarseness lasted from 5 to 11 days in four subjects. One subject reported wheezing for three weeks after exposure. Wheezing was absent on examination in three of the most symptomatic subjects. Spirometry was obtained in 60 subjects. Smoke exposure showed no effect on FEV1 or FVC, adjusted for cigarette smoking. There was no significant interaction between cigarette smoking and smoke exposure on FEV1 ( $p = 0.20$ ) but there was a borderline interaction for FVC ( $p = 0.05$ ). Results were as follows:

Smoking status	Low exposure to smoke [% predicted]		High exposure to smoke [% predicted]	
Current Smoker	FEV1	103.7	FEV1	96.5
	FVC	105.5	FVC	93.9
Non smoker	FEV1	97.8	FEV1	100.6
	FVC	94.2	FVC	96.4

## ADVERSE HEALTH EFFECTS FROM RED PHOSPHORUS SMOKE

### General Reviews / Comments

The US National Research Council produced a report on the toxicity of military smokes and obscurants.<sup>56</sup> The first volume (1997) dealt with four obscurant smokes; diesel fuel, fog oil, red phosphorus and hexachloroethane. Concerns expressed about the health effects of red phosphorus smoke included: respiratory tract irritation and inflammation in humans. Relevant sections of this Report are reproduced as follows:

*Red phosphorus smoke is deployed explosively from grenades and mortar shells. The obscurant portion of the grenades consists of a 95:5 mixture of red phosphorus and butyl rubber, which, when combusted, produces aerosols of phosphoric acid in a complex mixture of polymeric forms.*

*The obscurant portion of the grenades consists of a 95:5 mixture of red phosphorus and styrene-butadiene rubber (butyl rubber) in the presence of methylene chloride, which is later removed by low temperature drying (Lundy and Eaton 1994). Analyses of samples for methylene chloride found none present (Brazell et al 1984). The purpose of the butyl rubber is to reduce the cloud-pillar effect found with pure red phosphorus. This mixture of red phosphorus and butyl rubber (RP-BR) also contains two other compounds. The red phosphorus is coated with about 1.25% (by weight) of insulating oil, and approximately 1% talc or silica is added to break up and improve uniformity of the pattern.*

*Red phosphorus is also the major ingredient in mortar rounds used to generate smoke. In that use, it is combined with sodium nitrate and an epoxy binder in a ratio of 80:14:6 parts by weight, respectively.*

*The combustion products associated with RP-BR have been chemically and physically characterized by the U.S. Army. Table 4-1 summarizes the composition of phosphoric acids in RP-BR smoke. The combustion products of RP-BR and white phosphorus impregnated in felt, also used by the Army to generate an obscuring smoke, are similar under the same burn conditions (Ramsey et al 1985). The particles are composed primarily of various phosphoric acids present as a complex mixture of polymeric forms with organic compounds and inorganic gases only at trace amounts. Phosphorus trioxide is particularly likely to be formed, which is of interest because it reacts with water to form phosphoric acid and phosphine (Ballou 1981). The relative proportion of the different acids of phosphorus in RP-BR smoke changes with time after it is generated, but the predominant component of the smoke remains phosphoric acid (orthophosphate) (Ballou 1981; Mitchell and Burrows 1990). Only trace amounts of phosphine have been measured in some cases (Ballou 1981).*

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<sup>56</sup> National Research Council (1997). Toxicity of Military Smokes and Obscurants. Volume 1. Subcommittee on Military Smokes and Obscurants. National Academy Press: Washington, DC. Accessed at <http://www.nap.edu/openbook/0309057361/html>

TABLE 4-1 Composition of the Phosphoric Acids in  
RP-BR Smoke from Static Burn

Component	Composition %
Orthophosphate	22.8
Pyrophosphate	19.6
Tripolyphosphate	13.3
Tetrapolyphosphate	11.5
P <sub>5</sub> -P <sub>13</sub>	32.8
Higher polyphosphates	Low

Source: Brazell et al. (1984).

*The high phosphoric acid content of the smoke causes respiratory-tract irritation and inflammation in humans and animals at concentrations of 180 mg/ cubic metre. Inhalation of red phosphorus-butyl rubber smoke by rats produces terminal bronchiolar fibrosis. Induction of fibrosis appears to be influenced by both concentration and duration of exposure.*

*No studies have been conducted on the effects of RP-BR smoke in humans. However, Mitchell and Burrows (1990) estimated that human exposure to RP-BR at concentrations of about 2,000 mg/cubic metre for longer than 15 minutes might result in death. They suggested further that acute exposure at concentrations of 1,000 mg/cubic metre should be considered intolerable and that 700 mg/cubic metre is the highest tolerable concentration; above that, masks must be worn (Mitchell and Burrows 1990). Others have reported that concentrations exceeding 100 mg/cubic metre were unendurable for all workers except the "inured" worker (ACGIH 1991).*

*The most sensitive toxic effect following short-term exposures of humans and animals to red phosphorus-butyl rubber aerosols is respiratory distress. Concentrations as low as 100 mg/ cubic metre are considered to be intolerable to humans, even for short periods.*

*The PEGL [permissible exposure guidance level] recommended for red phosphorus-butyl rubber is based on the American Conference of Governmental Industrial Hygienist's (ACGIH) Threshold Limit Value (TLV) time-weighted average (TWA) for phosphoric acid, which is the primary combustion production of concern. The TLV-TWA of 1.0 mg per cubic metre appears to protect occupational workers adequately and, therefore, seems appropriate for military personnel as well.*

Several animal studies were discussed in this Report and are summarised here (also see tables 4.2 and 4.3 below). Residues of combusted RP-BR smoke instilled in the eyes of rabbits produced severe irritation and corneal ulceration. Residues of combusted RP-BR smoke applied to the skin of rabbits also produced severe irritation. Single acute inhalational exposure to RP-BR smoke produced conjunctivitis in rats and dogs and pulmonary effects in rats, guinea pigs and dogs [respiratory distress in all three animals; pulmonary oedema and haemorrhage, laryngeal oedema and erosion in rats]. Repeated inhalational exposure to RP-BR smoke produced:

- Transient reddening and swelling of the eyelids in rats.

- Wheezing and laboured breathing in rats during the period of exposure. Terminal bronchiolar fibrosis was observed in rats and this was evident after exposure at 400 mg per cubic metre for 3.5 hours per day for four consecutive days (Aranyi 1983). The fibrosis increased in incidence and severity with increasing exposure concentrations and duration and no recovery was observed over two weeks post exposure.
- Increased locomotor activity in rats with incomplete recovery two weeks following exposure.
- No effects were seen on testicular toxicity in rats.
- No developmental effects in rats apart from decreased birth weight, which recovered (Weimer et al 1980)
- Weak clastogenicity in the micronucleus test in the bone marrow and red blood cells of rats that were exposed eight times over a two week period at 1000 mg per cubic metre for 2.25 hours (Aranyi 1984). No long-term carcinogenicity studies in animals were found.

TABLE 4-2 Acute Lethality of Red Phosphorus-Butyl Rubber Smoke

Species	Exposure Duration	Exposure Concentration (mg/m <sup>3</sup> )	End Points and Comments	Reference
Rat	1 hr/d, 5 d	2,320	LC <sub>50</sub>	Aranyi et al. 1988a
Rat	4 hr/d, 5 d	≤1,000	No significant mortality	Aranyi et al. 1988a
Rat	1 hr	4,000	LC <sub>50</sub>	Ballou 1981; Shinn et al. 1985
Rat	1 hr	8,460	8/9 died with 2 d	Ballou 1981
Rat	1 hr	3,100	20% lethality	Ballou 1981
Rat	60 min	1,128	No deaths	Weimer et al. 1977
	120 min	1,676	4/10 died	
	150 min	1,625	5/10 died	
	180 min	1,572	8/10 died	
Guinea pig	5 min	120	No deaths	Weimer et al. 1977
	10 min	352	4/10 died	
	15 min	484	7/10 died	
	10 min	797	9/10 died	
Dog	≤240 min	≤1,882	No deaths	Weimer et al. 1977



TABLE 4-3 Summary of Nonlethal Effects of Exposure to Red Phosphorus-Butyl Rubber Smoke Aerosol

Category and Species	Exposure Frequency and Duration	NOAEL (mg/m <sup>3</sup> )	LOAEL (mg/m <sup>3</sup> )	End Point and Comments	Reference
Effects in Humans					
Human (workers)	8 hr/d, 5 d/wk, several years	—	100	Unendurable except for the "hardened" worker	ACGIH 1991
Effects in Animals					
One-Time Inhalation Exposures					
<i>Skin and Eye Irritation</i>					
Rat	180 min	—	1,813	Conjunctivitis	Weimer et al. 1977
Dog	240 min	—	1,882	Conjunctivitis	Weimer et al. 1977
<i>Pulmonary Effects</i>					
Rat	1 hr	—	3,150	Laryngeal and tracheal lesions; pulmonary edema (and some lethality)	Ballou 1981
Rat	4 hr	—	1,530	Laryngeal and tracheal lesions; pulmonary edema (and some lethality)	Ballou 1981
Rat	1 hr	—	1,128	Respiratory distress; hypoactivity; salivation	Weimer et al. 1977
Rat	3.5 hr, one time	—	1,000	Decrease from 80% (control) to 35% bactericidal activity; reversible in clean air (only one exposure concentration tested)	Aranyi et al. 1988b
Guinea pig	5 min	—	120	Respiratory distress; hypoactivity; salivation	Weimer et al. 1977
Dog	30 min	—	1,519	Respiratory distress; hypoactivity; salivation	Weimer et al. 1977
Dog	90 min	—	1,212	Respiratory distress; hypoactivity; salivation	Weimer et al. 1977
<i>Other Effects</i>					
Rat	120 min	—	1,676	Reduced kidney weight (and some lethality)	Weimer et al. 1977
Rat	150 min	—	1,625	Reduced body weight (and some lethality)	Weimer et al. 1977
Repeated Inhalation and Ocular Exposures					
<i>Eye Irritation</i>					
Rat	8 min/d, 5 d/wk, 12 wk	—	22	Transient reddening and swelling of eyelids during 8th wk only	Weimer et al. 1980
<i>Pulmonary Effects</i>					
Rat (Sprague-Dawley and Fischer)	8 min/d, 5 d/wk, 12 wk	165	—	No effects	Weimer et al. 1980
Mice (Swiss and A strain)	8 min/d, 5 d/wk, 12 wk	165	—	No effects	Weimer et al. 1980
Guinea pig	8 min/d, 5 d/wk, 12 wk	165	—	No effects	Weimer et al. 1980

TABLE 4.3 (Continued)

Category and Species	Exposure Frequency and Duration	NOAEL (mg/m <sup>3</sup> )	LOAEL (mg/m <sup>3</sup> )	End Point and Comments	Reference
Rabbit	8 min/d, 5 d/wk, 12 wk	165	—	No effects	Weimer et al. 1980
Rat	3.5 hr, 4 d	—	400	Terminal bronchiolar fibrosis	Aranyi 1983
Rat	2.25 hr/d, 4 d/wk, 4 wk	♀: 750 ♂: 1,000	♀: 1,000 ♂: 1,200	Increased protein in lavage fluid	Aranyi 1984
Rat	2.25 hr/d, 4 d/wk, 4 wk	400	750	Terminal bronchiolar fibrosis	Aranyi 1984
Rat	2.25 hr/d, 4 d/wk, 13 wk	—	300	Decreased total cells in pulmonary lavage fluid; reversible in clean air	Aranyi et al. 1988b
Rat	2.25 hr/d, 4 d/wk, 13 wk	180	300	Reduced bactericidal activity; reversible in clean air	Aranyi et al. 1988a,b
Rat	2.25 hr/d, 4 d/wk, 13 wk	50	180	Terminal bronchiolar fibrosis	Aranyi 1986; Aranyi et al. 1988a
<i>Biochemical Effects</i>					
Rat	2.25 hr/d, 4 d/wk, 4 wk	—	♀: 400 ♂: 750	Decrease in cholesterol and BUN levels	Aranyi 1984
<i>Immunological Effects</i>					
Rat	2.25 hr/d, 4 d/wk, 4 wk	400	750	Decreased white-blood-cell count	Aranyi 1984
Rat	2.25 hr/d, 4 d/wk, 4 wk	—	♀: 400 ♂: 750	Increased cellular ATP levels	Aranyi 1984
Rat	2.25 hr/d, 4 d/wk, 4 wk	—	750	Decreased activity of 5'-nucleotidase	Aranyi 1984; Aranyi et al. 1988b
Rat	2.25 hr/d, 4 d/wk, 13 wk	—	300	Increased cellular ATP levels	Aranyi et al. 1988b
Rat	2.25 hr/d, 4 d/wk, 13 wk	300	750	Decreased activity of 5'-nucleotidase	Aranyi et al. 1988b
<i>Behavioral Effects</i>					
Rat	2.25 hr/d, 5 d/wk, 4 wk	—	400	Increased motor activity; incomplete recovery after 2 wk in clean air	Aranyi 1983, 1984
<i>Reproductive and Developmental Effects</i>					
Rat	8 min/d, 5 d/wk, 10 wk	132	1,186	Decreased birth weight (reproductive end points not fully evaluated)	Weimer et al. 1980; Lundy and Eaton 1994
<i>Mutagenic Effects</i>					
Rat	2.25 hr/d, 4 d/wk, 2 wk	—	1,000	Clastogenic response	Aranyi 1984

Abbreviations: hr, hour(s); min, minute(s); d, day(s); wk, week(s); ♂, male; ♀, female.

Notes: Aranyi (1984) used exposure concentrations of 400, 750, and 1,000 mg/m<sup>3</sup> for females and 750, 1,000, and 1,200 mg/m<sup>3</sup> for males. Thus, if an effect was observed at all concentrations tested, the LOAEL (without a NOAEL) would be 400 mg/m<sup>3</sup> for females and 750 mg/m<sup>3</sup> for males. Aranyi (1983, 1984) and Aranyi et al. (1988a,b) found no differences in responses between male and female rats exposed for 2.25 hr/d, 4 d/wk for 4 wk; therefore, only male rats were used in the 13-wk exposure experiments.

The Tenth Report on Carcinogens, by the US National Toxicology Program, did not list red phosphorus or red phosphorus smoke as an agent known to be a human carcinogen or reasonably anticipated to be a human carcinogen.<sup>57</sup>

Review of the database of the International Agency for Research on Cancer revealed no entry regarding an evaluation of the carcinogenicity of red phosphorus or red phosphorus smoke.

Red phosphorus smoke was recently reviewed (2002).<sup>58</sup> The authors noted that red phosphorus was not as reactive as white phosphorus. Red phosphorus reacted slowly with atmospheric moisture and the smoke did not produce thermal injury, hence red phosphorus smoke was less toxic.

The ChemWatch summary for red phosphorus included risk statements of highly flammable, explosive when mixed with oxidising substances, and very toxic and harmful to aquatic organisms.<sup>59</sup> No specific references were made to humans.

Ballantyne (1998) studied the acute inhalation toxicity of red phosphorus smoke in animals.<sup>60</sup> The author commented that the marked species differences in lethal toxicity suggested caution in extrapolation to humans. However, he stated that overexposure certainly would result in acute irritant and corrosive effects on the respiratory tract, and long-term repeated exposures could produce chronic pulmonary disease. He noted a study where exposure of human volunteers to concentrations of 100-700 mg per cubic metre for 2 to 15 minutes caused significant, but reversible, symptoms of respiratory distress with eye irritation (Uhrmacher et al 1985 - A Health and Effects Database Assessment of US Army Waste Material).

### *Phosphoric Acid*

Both the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) have a permitted or recommended exposure limit respectively, of 1 mg per cubic metre, for phosphoric acid.<sup>61 62 63</sup> NIOSH noted that acute inhalational exposure to phosphoric acid was associated with irritation of the upper respiratory tract [burning sensation, sore throat, cough, and shortness of breath]. Acute skin exposure to phosphoric acid was associated with irritation of skin [redness, pain, blisters, burns, and dermatitis]. Acute exposure of phosphoric acid to eyes was associated with eye irritation [pain and redness, burns]. Acute ingestion of phosphoric acid was also corrosive [abdominal

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<sup>57</sup> US National Toxicology Program. Tenth Report on Carcinogens. US Department of Health and Human Services. Accessed at <http://ehp.niehs.nih.gov/roc/toc10.html> on 16<sup>th</sup> July 2003

<sup>58</sup> Anon (2002). Smokes, fuels, and incendiary materials. J R Army Med Corps 148: 395-7. ID 28186.

<sup>59</sup> ChemWatch Summary for Red Phosphorus. Accessed at <http://dsmachem.dcb.defence.gov.au> On 15<sup>th</sup> May 2003

<sup>60</sup> Ballantyne B (1998). Acute inhalation toxicity of red phosphorus smoke. Toxic Substance Mechanisms 17: 251-266. ID 28305

<sup>61</sup> Occupational Safety and Health Administration. Chemical Sampling Information for Phosphoric Acid. Accessed at [www.osha.gov/dts/chemicalsampling/data/CH\\_262500.html](http://www.osha.gov/dts/chemicalsampling/data/CH_262500.html). On 17<sup>th</sup> July 2003.

<sup>62</sup> National Institute for Occupational Safety and Health. Pocket Guide to Chemical Hazards - Phosphoric Acid. Accessed at [www.cdc.gov/niosh/npg/npgd0506.html](http://www.cdc.gov/niosh/npg/npgd0506.html) On 17<sup>th</sup> July 2003

<sup>63</sup> National Institute for Occupational Safety and Health. International Chemical Safety Cards - Phosphoric Acid. [www.cdc.gov/niosh/ipcsneng/neng1008.html](http://www.cdc.gov/niosh/ipcsneng/neng1008.html) On 17<sup>th</sup> July 2003

pain, burning sensation, shock or collapse]. NIOSH noted that human data indicated that phosphoric acid does not cause any systemic effect and the risk of pulmonary oedema from mist or spray inhalation was very remote [from chemical safety data sheet 1958].

The IRIS database (Integrated Risk Information System of the US Environmental Protection Agency) reviewed phosphoric acid.<sup>64</sup> In this review, it mentioned that the occupational study of Dutton et al. (1993) had some relevance to phosphoric acid because levels of phosphorus oxidation products were reported. The study examined lung function in a cohort of 131 workers involved in refining phosphorus rock to obtain elementary phosphorus. Years of exposure, which were estimated from work records based on where workers spent their entire working days, ranged from 0-46 years, with a mean of 11.4 years. The study indicated that the maximum level of phosphorus oxidation products (as phosphorus pentoxide) was measured at 2.23 mg/cubic metre. Pulmonary function tests (forced vital capacity, forced expiratory volume in 1 second, and forced expiratory flow) were conducted annually over an 8-year period in all workers. These data were analysed longitudinally and cross-sectionally over the third through the seventh year of exposure. Neither analysis revealed any significant residual effect after adjusting for age and smoking. Although this study had several limitations, these data did indicate that long-term exposures to levels of airborne phosphorus oxidation products over twice the recommended ACGIH (1991) 8-hour TLV had no demonstrable effect on lung function. The relevance of this study to red phosphorus smoke is less clear.

### ***Animal Studies***

Burton et al (1982) studied the inhalation toxicity of RP-BR smoke in Sprague-Dawley rats.<sup>65</sup> Groups of ten rats were exposed to single one hour periods at four different concentrations (3.1, 4.3 5.3 and 8.5 mg/L) of RP-BR smoke and to a single four hour exposure at 1.5 mg/L. There was no control group. Rats were observed for 14 days after exposure and gross autopsy was performed, without histological examination. The smoke was generated from combustion of a 95:5 mixture of red phosphorus and butyl rubber with 1% mineral oil and 1% talc. Chemical analysis suggested that the principal product in the combusted smoke was phosphorus pentoxide, which then hydrolysed to form a series of polyphosphoric and cyclopolyphosphoric acids. These included phosphoric (predominant), diphosphoric, triphosphoric, cyclotetraphosphoric, cyclotriphosphoric and tetraphosphoric acids. Trace amounts of phosphine in the smoke were also detected (at 2 ppm). There was no evidence of white phosphorus in the smoke. Rats died from the end of exposure to 11 days after exposure and death occurred at all five smoke concentrations. At all concentrations of the RP-BR smoke, epiglottal and laryngeal lesions were present in deceased rats. The severity of the lesions and the number of rats affected appeared to increase with the concentration of the smoke. Epiglottis and laryngeal lesions consisted of ulceration, oedema, and fibrinous exudate. Surviving rats also had epiglottis deformations. In general, the nares, turbinates and eyes appeared grossly

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<sup>64</sup> Integrated Risk Information System, US Environmental Protection Agency. Phosphoric Acid. Accessed at <http://toxnet.nlm.nih.gov> on 21th July 2003

<sup>65</sup> Burton F, Clark M, Miller R, et al (1982). Generation and characterization of red phosphorus smoke aerosols for inhalation exposure of laboratory animals. Am Ind Hyg Assoc 43: 767-772. ID 28093

unaffected. Lung congestion, oedema and haemorrhage were present at the two higher smoke concentrations.

Marrs et al (1984) studied the inhalation toxicity of red phosphorus smokes in rabbits and rats exposed to a single inhalation of 30 minutes.<sup>66</sup> 10 New Zealand female white rabbits and 10 female Porton-Wistar derived rats were exposed to a smoke produced from burning either a mixture of red phosphorus and butyl rubber (95:5) or red phosphorus and butadiene styrene (97:3). These smokes produced a solid material mass of 3.2 and 3.1 g per cubic metre respectively [0.68 and 0.67 g per cubic metre as phosphorus, respectively]. Five rabbits and five rats served as a control group for each exposed group. Animals were observed for up to 14 days after exposure and autopsies with histological examination were performed 24 hours or 14 days after exposure. None of the rabbits died with either red phosphorus smoke and histological lesions detected consisted mainly of laryngeal and tracheal inflammation, alveolitis and in a few cases bronchopneumonia. One rat died during exposure to the RP-BR smoke and four died within 24 hours of the RP-BS smoke. Histological lesions were similar in rats except that two of the deceased rats had pulmonary oedema. Pulmonary congestion was also observed in the rats. The control animals were normal except that two of ten rabbits had mild alveolitis.

Marrs et al (1989) studied the toxicity of repeated inhalation of a red phosphorus smoke in mice, rats, and guinea pigs.<sup>67</sup> The red phosphorus smoke used was generated from the combustion of a mixture of oiled red phosphorus (95%) and polyvinyl butyral BL18 (5%). Animals were exposed to two concentrations of smoke; 16-17 mg per cubic metre and 128 mg per cubic metre, as phosphorus. These exposures lasted for one hour per day, five days per week. The mice received 180 exposures while the other animals received 200 exposures (except for the high-dose guinea pig group). 100 mice each were in the control group, low dose group and high dose group. 50 rats each were in the three respective groups while 48 guinea pigs each were in the control and low dose group and 42 in the high dose group. Autopsies with histological examination were conducted. Animals were observed for up to 19 months after the start of exposure. Survival in rats was similar between the exposed groups and the control group. Amongst mice, the death rate was similar between the control group (59%) and the low dose group (63%) but was higher in the high dose group (78%). Amongst guinea pigs, the death rate was lowest in the control group (15%), intermediate in the low dose group (38%) while all guinea pigs in the high dose group (100%) died during or immediately after their first exposure to the smoke. With the exception of the high-dose guinea pig group, deceased animals failed to show pathological changes likely to have been a consequence of smoke exposure. In the high-dose guinea pig group, severe lung congestion was present in all deceased animals and considered the result of smoke exposure. The growth of exposed mice and rats relative to controls was depressed during the exposure period. In all three species, scanty changes were observed in surviving animals and these mostly appeared unrelated to smoke exposure. An exception was the significantly more frequent presence of alveolar aggregates of macrophages with granules in the low- (9/37) and high- (9/22) dose mice compared to control mice (2/41). Chronic

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<sup>66</sup> Marrs T (1984). Histological changes produced by exposure of rabbits and rats to smokes produced from red phosphorus. *Toxicology Letters* 21: 141-146. ID 28185

<sup>67</sup> Marrs T, Colgrave H, Edginton J, et al (1989). The toxicity of a red phosphorus smoke after repeated inhalation. *Journal of Hazardous Materials* 22: 269-282. ID 28304

interstitial nephritis was significantly more common in the low-dose guinea pigs compared to control animals [21/30 versus 17/41,  $p < 0.05$ ], but the relationship to smoke exposure was uncertain. No significant differences were observed in the frequency of neoplasms between exposed and control animals for any of the three animal species. The authors noted that red phosphorus smoke was largely an aerosol of orthophosphoric acid and despite this, specific damage to the respiratory tract was rarely seen in their study. It was possible that damage present at the end of smoke exposure may have been reversed by the end of the observation period (and hence undetected).

Shumake et al (1992) studied the inhalation toxicity of RP-BR smoke, in two wildlife species [black-tailed-prairie-dogs (a rodent) and rock doves], exposed to 2.0-6.0 mg/L, one to four times for one hour.<sup>68</sup> Controls were exposed to filtered air. Animals were observed for up to 28 days post-exposure. Dead and surviving animals were autopsied on day 31. No deaths occurred in the prairie dogs but impaired vocalisation and respiratory congestion was seen with the higher concentration (lung congestion was confirmed at autopsy). In the rock dove group, ten of 24 males (41.7%) and one of 18 females (5.6%) died, on average 5.4 days after exposure. Surviving birds showed hunched postures, impaired vocalisation and respiratory congestion. Excessive mucus in the nasal passages and larynges of birds exposed to the higher concentration was found at autopsy.

Johns et al (1992) studied the inhalational toxicity of repeated exposure to RP-BR smoke, in two wildlife species [black-tailed-prairie-dogs and rock doves], exposed to 0, 1.0 and 4.0 mg/L, for 80 minutes, on four days.<sup>69</sup> There was no significant physiological effect on prairie dogs. An increased respiratory rate, followed by death, was observed in two rock doves, after two daily smoke exposures.

Sterner et al (1993) studied the inhalational toxicity of repeated exposure to RP-BR smoke, in two wildlife species [black-tailed-prairie-dogs and rock doves], exposed to 0, 1.0 and 4.0 mg/L, for 80 minutes, on two and four days.<sup>70</sup> The phosphoric acid concentrations in aerosols were 0.57 and 2.64 mg/L. Transient decreases in ingestive behaviours (food and water consumption) accompanied by decreases in body weight gain were seen in both animals. The authors considered that this may have resulted from transient irritation and ulceration of the oesophageal mucosa.

Shumake et al (1994) studied the inhalational toxicity of repeated exposure to RP-BR smoke, in two wildlife species [black-tailed-prairie-dogs and rock doves], exposed to 3.0 and 6.0 mg/L, for up to four days.<sup>71</sup> Transient weight loss was observed in prairie dogs. While a severe protracted weight loss was observed in rock doves exposed to

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<sup>68</sup> Shumake S, Sterner R, Johns B, et al (1992). Qualitative effects associated with red phosphorus smoke inhalation exposures in two wildlife species: prairie dogs and rock doves. (Abstract). *Inhalation Toxicology* 4: 411-431.

<sup>69</sup> Johns B, Thompson R, Crane K (1992). Physiological effects of red phosphorus smoke inhalation on prairie dogs and rock doves. (Abstract). *Environ Toxicol Chem* 11: 1345-1353.

<sup>70</sup> Sterner R (1993). Whole-body exposures to a phosphoric acids aerosol: II. Food/water/weight effects in wild rodent and avian species. (Abstract). *Journal of Toxicology and Environmental Health* 39: 497-515.

<sup>71</sup> Shumake S, Sterner R, Engeman R (1994). Changes in body weight and water consumption in black-tailed prairie dogs and rock doves following red phosphorus smoke exposures. (Abstract). *Inhalation Toxicology* 6: 407-25.

the higher concentration and this weight loss was not recovered over the 28-day study period.

Ballantyne (1998) studied the acute inhalation toxicity of red phosphorus smoke in male rabbits, rats, mice and guinea pigs.<sup>72</sup> Red phosphorus smoke was generated from ignition of pure unformulated red phosphorus. Animals were exposed for one hour to varying concentrations of the smoke [rabbits and rats 450-2130, mice 111-870 and guinea pigs 36-450 mg per cubic metre, as phosphorus]. Four groups of ten rabbits each, four groups of between 9 to 12 rats each, five groups of 20 or 50 mice each and four groups of 10 or 20 guinea pigs each were utilised. Survivors were sacrificed 14 days after exposure and all animals underwent autopsy. Most animals that died did so during exposure, although a small proportion survived a few hours or days following exposure. There was a marked species difference in the susceptibility to the smoke. The one-hour LC-50 was 1689 for rabbits, 1217 for rats, 271 for mice, and 61 for guinea pigs (mg per cubic metre, as phosphorus). In rabbits, rats and mice that died, there were similar pathological findings in the respiratory tract. These consisted of laryngotracheal epithelial or mucosal necrosis and acute inflammatory cell infiltration, pulmonary congestion and oedema, alveolar haemorrhages, alveolar wall polymorphonuclear cell infiltration, bronchiolitis, and macrophage aggregations in alveoli and bronchioles. The author considered that these findings were consistent with respiratory tract injury from the corrosive effects of phosphoric acids in the smoke. Guinea pigs at the highest lethal concentrations showed no lesions in the larynx and trachea and only alveolar capillary congestion in the lungs. The author felt that these findings were consistent with asphyxia secondary to laryngospasm in guinea pigs. Surviving rabbits, rats and mice showed residual laryngotracheal necrosis and inflammation in a few instances. There was pneumonitis evident in some surviving rabbits and rats exposed to the two higher smoke concentrations while lung congestion was present in many surviving mice. Surviving guinea pigs had mild laryngotracheal necrosis and inflammation, with occasional pulmonary congestion, oedema, and inflammatory infiltration. The author considered that the minimal findings in survivors from potential lethal exposure concentrations indicated that some reversibility and healing occurs, although the necrotic nature of the lesions may predispose to secondary infection. The author also commented that the marked species differences in lethal toxicity suggested caution in extrapolation to humans.

Henry et al (1982) evaluated the acute toxicity of a red phosphorus sample that contained oil in several mammalian species.<sup>73</sup> The sample did not produce irritation in the eyes of rabbits at a dose of 100 mg. It was non-irritating to intact or abraded skin of rabbits when applied at doses of 0.5 g per application site under a patch for 24 hours. Dermal application to guinea pigs did not produce skin sensitisation and intradermal treatment produced slight irritation but not sensitisation. The oral LD50 in rats was greater than 10g/kg. This study appeared to evaluate red phosphorus per se rather than red phosphorus smoke.

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<sup>72</sup> Ballantyne B (1998). Acute inhalation toxicity of red phosphorus smoke. Toxic Substance Mechanisms 17: 251-266. ID 28305

<sup>73</sup> Henry M, Barkley J, Rowlett C (1982). Mammalian toxicologic evaluation of hexachloroethane smoke mixture and red phosphorus. (Abstract). Report number AD-A109. Litton Bionetics Inc, Kensington, MD.

## ADVERSE HEALTH EFFECTS FROM WHITE PHOSPHORUS SMOKE

### General Reviews / Comments

The US National Research Council produced a report on the toxicity of military smokes and obscurants.<sup>74</sup> Volume 2 dealt with four obscuring smokes; white phosphorus, brass, titanium dioxide and graphite. Some adverse health effects were noted with white phosphorus smoke. Namely: nasal and throat irritation, cough, tightness of chest and dyspnoea in human volunteers after acute exposure. Acute bronchitis and laryngitis were also mentioned.

Elemental white phosphorus was also discussed (as opposed to white phosphorus smoke). Oral ingestion of white phosphorus in humans was considered highly toxic, resulting in widespread organ damage and even death. Fatal or near-fatal human exposures have occurred as a consequence of oral ingestion during a suicide attempt or as a consequence of dermal burns during munitions explosions. Long term occupational exposure to airborne phosphorus vapours can result in necrosis of the jaw and oral cavity in some workers.

Relevant sections of this Report concerning white phosphorus smoke are reproduced as follows:

*White phosphorus (WP) smoke is used by the military in mortar and artillery shells and grenades to block the transmission of visible light, infrared light, or microwaves. It is the most effective obscuring smoke to defeat thermal imagery systems. Phosphorus smoke is generated from a phosphorus-containing flammable matrix that burns to form solid particles of phosphorus pentoxide ( $P_2O_5$ ) in air.  $P_2O_5$  reacts with moisture to form orthophosphoric acid ( $H_3PO_4$ ). In this report, toxicity data and exposure guidance levels for WP smoke are reported as  $H_3PO_4$  equivalents.*

*When munitions containing WP are fired, they burn and produce smoke. The combustion of WP will produce smoke made up of various oxides of phosphorus, including  $P_2O_5$  and phosphorus trioxide ( $P_4O_6$ ). These oxides react rapidly with moisture to form a number of transformation products, such as  $H_3PO_4$  and pyrophosphoric acid ( $H_4P_2O_7$ ) (Table 2-1; Brazell et al 1984) and about 10% unburned phosphorus (Spanggord et al 1985, ATSDR 1997). Organic compounds (concentrations in parts per billion) and some inorganic gases might be present, but only at trace levels. Because WP is not likely to persist long in air, a majority of phosphorus compounds released and dispersed in air during military use of smokes are likely to be deposited as phosphoric acid or phosphates on land and water (EPA 1990). The smoke particle diameter is about 1 micrometer ( $\mu m$ ) by count, 98% of the particles are below 2  $\mu m$  in diameter (Katz et al 1981).*

*The chemical characteristics of WP and red-phosphorus-butyl rubber(RP-BR) smokes are similar; both are primarily phosphoric acids, present as a complex mixture of polymeric forms.*

<sup>74</sup> National Research Council (1999). Toxicity of Military Smokes and Obscurants. Volume 2. Subcommittee on Military Smokes and Obscurants. National Academy Press: Washington, DC. Accessed at <http://www.nap.edu/openbook/0309063299/html>



**TABLE 2-1 Composition of Phosphoric Acids in White Phosphorus Smokes  
Produced from Static Burn**

Component	Chemical Formula	Composition by weight (%)
Orthophosphoric acid	$H_3PO_4$	23.8
Pyrophosphate	$H_4P_2O_7$	26.6
Triphosphate	$H_5P_3O_{10}$	16.3
Tetraphosphate	$H_6P_4O_{13}$	11.3
$P_5-P_{13}$	$H_{n+2}P_nO_{3n+1}$	22.0
Higher polyphosphates	$H_{n+2}P_nO_{3n+1}$	Low

Source: Brazell et al. 1984.

*The subcommittee recognizes that these EEGLs are lower than those recommended by this subcommittee for RP-BR (NRC 1997), even though the final combustion products for both smokes are expected to be phosphoric acid. However, the human and animal data indicate that WP smoke appears to produce respiratory irritation at lower concentrations than does RP-BR smoke. For example, the rats exposed for 1 hr to RP-BR showed signs of respiratory irritation with  $H_3PO_4$  at 1,692 mg per cubic metre, but none died (Weimer et al 1977). Rats exposed to WP smoke, with  $H_3PO_4$  at 1,794 mg per cubic metre, a concentration similar to that of RP-BR, had a 20% mortality during the 1-hr exposure (Brown et al 1980). The difference might result from the presence of some uncombusted WP in the WP smoke. That would contribute to the smoke's toxicity.*

*A similar difference in sensitivity to the two phosphorus smokes can be observed when comparing the human data on WP and RP-BR smokes. For example, Mitchell and Burrows (1990) stated that acute exposure to RP-BR smoke at 1,000 mg per cubic metre (chemical form not reported) would be intolerable and that 700 mg per cubic metre (chemical form not reported) is the highest tolerable concentration. In contrast, White and Armstrong (1935) stated that human volunteers exposed to WP smoke with  $P_2O_5$  at 592 mg per cubic metre ( $H_3PO_4$  at 818 mg per cubic metre) said that was the limit of their tolerance.*

*The most sensitive toxic response to acute exposure (one exposure or multiple exposures occurring within a short time, usually 24 hr or less) to WP smoke is respiratory irritation and distress. Such an effect became evident in goats and rats following a 1-hr exposure to  $H_3PO_4$  at 745 and 525 milligrams per cubic meter (mg/m<sup>3</sup>), respectively. Human volunteers exposed for 3.5 min to  $H_3PO_4$  at 818 mg/m<sup>3</sup> reported respiratory irritation, tightness of the chest, cough, and difficulty in breathing.*

Relatively little information has been reported on human responses to inhalation of WP smoke. Exposure of 108 men to WP smoke at 87 1,770 mg/m<sup>3</sup> resulted in coughing and irritation of the throat (Cullumbine 1944, as cited in Wasti et al. 1978). The method used to measure the smoke concentration and the length of exposure were not reported. From those data, Cullumbine (1944, as cited in Wasti et al. 1978) estimated that the minimal exposure concentration causing coughing and throat irritation is about 700 mg/m<sup>3</sup> for working individuals and 1,000 mg/m<sup>3</sup> for individuals at rest.

A number of studies were conducted by White and Armstrong in 1935 with human volunteers. In most of those studies, the individuals were placed in a chamber, and then WP smoke was introduced. Male subjects were exposed to WP smoke with average concentrations of P<sub>2</sub>O<sub>5</sub> at 188-514 mg/m<sup>3</sup> for 2 to 15 min (White and Armstrong 1935). At the lowest concentration (P<sub>2</sub>O<sub>5</sub> at 188 mg/m<sup>3</sup> or H<sub>3</sub>PO<sub>4</sub> at 259 mg/m<sup>3</sup>), a 5-min exposure resulted in 50% of the individuals reporting respiratory distress, coughing, congestion, and throat irritation. At the highest concentration (P<sub>2</sub>O<sub>5</sub> at 514 mg/m<sup>3</sup> or H<sub>3</sub>PO<sub>4</sub> at 710 mg/m<sup>3</sup>), a 15-min exposure resulted in all subjects reporting tightness in the chest, coughing, nose irritation, and difficulty in speaking. The authors stated that exposure at an average concentration of P<sub>2</sub>O<sub>5</sub> at 514 mg/m<sup>3</sup> (H<sub>3</sub>PO<sub>4</sub> at 710 mg/m<sup>3</sup>) approaches the maximum concentration that can be tolerated for 15 min without serious effects. White and Armstrong (1935) stated that the concentration reported for the studies did not represent the maximum concentration to which the subjects were exposed, but instead represented an average of the concentration measurements taken throughout the exposure period. Thus, the maximum concentration in the chamber must have been considerably higher than the average concentration reported. For that reason, the White and Armstrong studies were not used in recommending guidance levels.

White and Armstrong (1935) conducted two additional studies in which the volunteers entered the chamber after the WP smoke concentration reached the desired level. In one study, a 2-min exposure of P<sub>2</sub>O<sub>5</sub> at 588 mg/m<sup>3</sup> (H<sub>3</sub>PO<sub>4</sub> at 812 mg/m<sup>3</sup>) resulted in coughing, tightness in the throat, and headaches. One individual developed acute bronchitis. In the second study, six volunteers were exposed for 3.5 min at a concentration of P<sub>2</sub>O<sub>5</sub> at 592 mg/m<sup>3</sup> (H<sub>3</sub>PO<sub>4</sub> at 818 mg/m<sup>3</sup>). The effects re-

ported were similar to those reported for the 2-min exposure. All effects were reversible.

An accidental exposure of four females to WP smoke in a closed room for 15-20 min (concentration not reported) resulted in numerous respiratory symptoms (i.e., nose and throat irritation), edema of larynx and vocal cords, and coughing. Injury apparently extended into the bronchi. Chest X-rays revealed patchy areas of infiltration that later cleared; however, laryngitis persisted for several months (Walker et al. 1947).

Five males were exposed to WP smoke composed of phosphorus at 35 mg/m<sup>3</sup> and P<sub>2</sub>O<sub>5</sub> at 22 mg/m<sup>3</sup> for 2 to 6 hr at 7-hr intervals, equivalent to H<sub>3</sub>PO<sub>4</sub> at 140 mg/m<sup>3</sup> (total exposure time not given). Within 6 to 20 hr, all developed symptoms of weakness, dry cough, headaches, tracheobronchitis, rales, tender and enlarged liver, and evidence of leukocytosis with relative lymphocytopenia (Aizenshtadt et al. 1971, as cited in Wasti et al. 1978). Erythrocyte acetylcholinesterase was reduced by 17%, and plasma acetylcholinesterase was reduced by 35%.

No deaths were reported in humans exposed to WP smoke with H<sub>3</sub>PO<sub>4</sub> at concentrations as high as 817 mg/m<sup>3</sup> (P<sub>2</sub>O<sub>5</sub> at 592 mg/m<sup>3</sup>) for 3 to 5 min or with H<sub>3</sub>PO<sub>4</sub> at 709 mg/m<sup>3</sup> (P<sub>2</sub>O<sub>5</sub> at 514 mg/m<sup>3</sup>) for 15 min (White and Armstrong 1935).

There are no data on gastrointestinal, cardiovascular, musculoskeletal, hepatic, renal, dermal and ocular, immunological, neurological, reproductive, developmental, genotoxic, or carcinogenic effects from inhalation of WP smoke by humans.

TABLE 2-3 Nonlethal Effects of White Phosphorus Smoke (Expressed as H<sub>3</sub>PO<sub>4</sub>) via Inhalation Exposure

Species	Exposure Duration	Exposure Concentration (mg/m <sup>3</sup> )	NOAEL (mg/m <sup>3</sup> )	LOAEL (mg/m <sup>3</sup> )	End Point and Comments	Reference
Respiratory Effects						
Human	Not specified	87-1,770	—	700 1,000	Coughing, respiratory irritation Irritation intolerable	Wasti et al. 1978; EPA 1990; ATSDR 1997
Human	2 min	812	—	812	Coughing, tightness in throat, and headaches; one developed acute bronchitis	White and Armstrong 1935
Human	3.5 min	818	—	818	Same symptoms as 812 mg/m <sup>3</sup> for 2-min exposure.	White and Armstrong 1935
Human	5 min	259-710 (average)	—	259 (average)	50% indicated sore throat, irritation, coughing, and congestion	White and Armstrong 1935
Human	15 min	259-710 (average)	—	710 (average)	All experienced tightness of chest, difficulty speaking; sug- gested maximum concentration without serious effects	White and Armstrong 1935
Human	15-20 min	Not specified	—	—	Choking, tightness in chest, rales, sore throat, sputum production	Walker et al. 1947; EPA 1990
Rat	60 min	758-3,030	—	758 3,030	Respiratory distress Intense congestion, edema, hemorrhages	Brown et al. 1980*

Rat	60 min	525-6,640	—	525	Pulmonary congestion, hemorrhages, respiratory distress, unmistakable signs of irritation	White and Armstrong 1935 <sup>b</sup>
Rat	90 min	1,200	—	1,200	Grasping, ataxia, respiratory distress	Brown et al. 1980 <sup>a</sup>
Guinea pig	10 min	138, 812, 984	138	—	No respiratory effects	Brown et al. 1980 <sup>a</sup>
Mouse	60 min	152-2,330	—	152	Unmistakable signs of irritation, congestion, difficulty breathing	White and Armstrong 1935 <sup>b</sup>
Goat	60 min	745-15,840	—	745	Unmistakable signs of irritation, inflammation, pneumonia	White and Armstrong 1935 <sup>b</sup>
Rat	15 min/d, 5 d/wk, for 13 wk	280, 884, 1,742	280	884, 1,742	Slight tracheitis and laryngitis Moderate-to-severe tracheitis and laryngitis	Brown et al. 1981 <sup>a</sup>
Hepatic Effects						
Rat	60 min	525-6,640	—	≥1,615	Slight clouding and swelling of liver in 1 rat; seen more consistently ≥3,494 mg/m <sup>3</sup>	White and Armstrong 1935 <sup>b</sup>
Rat	90 min	3,030	—	3,030	Liver congestion	Brown et al. 1980 <sup>a</sup>
Rat	15 min/d, 5 d/wk, 13 wk	289-1,742	1,742	—	No gross or histological changes	Brown et al. 1981 <sup>a</sup>
Guinea pig	10 min	138, 812, 984	984 <sup>c</sup>	—	No gross or histological changes	Brown et al. 1980 <sup>a</sup>

23

TABLE 2-3 (Continued)

Species	Exposure Duration	Exposure Concentration (mg/m <sup>3</sup> )	NOAEL (mg/m <sup>3</sup> )	LOAEL (mg/m <sup>3</sup> )	End Point and Comments	Reference
Guinea pig	30 min	192	192	—	No gross or histological changes	Brown et al. 1980 <sup>a</sup>
Mouse	60 min	152-2,330	—	649	Slight swelling and clouding of liver	White and Armstrong 1935 <sup>b</sup>
Goat	60 min	10,104	—	10,104	Slight swelling and clouding of liver	White and Armstrong 1935 <sup>b</sup>
Renal Effects						
Rat	60 min	525-6,640	—	≥1,615	Slight swelling and clouding of kidney	White and Armstrong 1935 <sup>b</sup>
Rat	90 min	3,030	—	—	No gross or histological changes	Brown et al. 1980 <sup>a</sup>
Rat	15 min/d, 5 d/wk, 13 wk	289, 884, 1,742	1,742	—	No gross or histological changes	Brown et al. 1981 <sup>a</sup>
Guinea pig	10 min	138, 812, 984	984 <sup>c</sup>	—	No gross or histological changes	Brown et al. 1980 <sup>a</sup>
Guinea pig	30 min	192	192	—	No gross or histological changes	Brown et al. 1980 <sup>a</sup>
Mouse	60 min	152-2,330	428	649	Slight swelling and clouding of kidney	White and Armstrong 1935 <sup>b</sup>

Goat	60 min	10, 104	—	10, 104	Slight swelling and clouding of kidney	White and Armstrong 1935 <sup>a</sup>
Reproductive and Developmental Effects						
Rat (male)	15 min/d, 5 d/wk, 10 wk	884,1,742	884	—	4 of 18 rats died in the high-dose group; no deaths in the low-dose group; no other significant effects observed	Starke et al. 1982
Rat (female)	15 min/d, gestation d 6-15	884,1,742	884	—	Increase in incidence of ectopic testes and reversed ductus arteriosus in the high-dose group; no effects in the low-dose group	Starke et al. 1982
Rat (male and female)	Males: 15 min/d, 5 d/wk, 10 wk before mating; Females: 15 min/d, 5 d/wk, 3 wk before mating and through gestation and lactation to d 21	884,1,742	884	—	Offspring body weight, survivability, and viability reduced in high-dose group; no significant effect on number of pups per litter and no abnormalities observed in either exposure group	Starke et al. 1982
<sup>a</sup> Reported value multiplied by 1.5 to correct for acid dissociation at pH of 9.6 to estimate H <sub>3</sub> PO <sub>4</sub> equivalents. <sup>b</sup> Originally reported as P <sub>2</sub> O <sub>5</sub> equivalents; multiplied by 1.381 to estimate H <sub>3</sub> PO <sub>4</sub> equivalents. <sup>c</sup> Highest NOAEL for a given species and study when exposure expressed as C x T. Abbreviations: NOAEL, no-observed-adverse-effect level; LOAEL, lowest-observed-adverse-effect level; C x T, the product of exposure concentration and time.						

The US Agency for Toxic Substances and Disease Registry (1997) produced a toxicological profile for white phosphorus that included white phosphorus smoke.<sup>75</sup> It noted that because white phosphorus smoke contains a number of phosphorus compounds and a small amount of white phosphorus, the toxicity of white phosphorus smoke can not be extrapolated from human and animal studies involving exposure to white phosphorus. Relevant sections of this profile are reproduced as follows:

*White phosphorus smoke is generated by burning white phosphorus. The U.S. Army uses white phosphorus smoke as a smoke/obscurant for training and testing activities. The smoke generated from burning white phosphorus consists primarily of oxidation and hydrolysis products of phosphorus, including phosphorus pentoxide and phosphorus trioxide. The moisture in the air reacts with these phosphorus oxides to produce a dynamic mixture of polyphosphoric acids that eventually transform into orthophosphoric acid, pyrophosphoric acid, and orthophosphorus acid. Wind-tunnel tests in which white phosphorus was burned and oxygen was non-limiting produced an average aerosol mass concentration between 2,500 and 3,000 mg per cubic metre, with the major components being polyphosphates, phosphine, and elemental phosphorus (Van Voris et al 1987). It should be stressed that while residual-coated white phosphorus is very biologically toxic, there are somewhat stable combustion intermediates (linear and cyclic polyphosphates) that can be persistent under low oxygen conditions and may be toxic to biological organisms.*

*There is limited information on the toxicity of white phosphorus smoke. Based on this information, the respiratory tract appears to be the most sensitive target. Because white phosphorus smoke contains a number of phosphorus compounds*

<sup>75</sup> ATSDR (1997). Toxicological Profile - White Phosphorous. Accessed at <http://www.atsdr.cdc.gov/toxprofiles/tp103.html>

*and a small amount of white phosphorus, the toxicity of white phosphorus smoke cannot be extrapolated from human and animal studies involving exposure to white phosphorus.*

### *Death*

*No deaths were reported in humans inhaling white phosphorus smoke at concentrations as high as 592 mg phosphorus pentoxide equivalents/m<sup>3</sup> (817 mg orthophosphoric acid equivalents/m<sup>3</sup>) for 3.5 minutes or 514 mg pentoxide equivalents/m<sup>3</sup> (709 mg orthophosphoric acid equivalents/m<sup>3</sup>) for 15 minutes (White and Armstrong 1935). In animals exposed to white phosphorus smoke, deaths have been observed following acute- and intermediate-duration inhalation exposure or acute oral exposure. The lowest lethal concentrations identified in animals exposed once to white phosphorus smoke are 1,943 mg orthophosphoric acid equivalents/m<sup>3</sup> for rats (Brown et al. 1980), 310 mg phosphorus pentoxide equivalents/m<sup>3</sup> (428 mg orthophosphoric acid equivalents/m<sup>3</sup>) for mice (White and Armstrong 1935), 677 mg orthophosphoric acid equivalents/m<sup>3</sup> for guinea pigs (Brown et al. 1980), and 6,230 mg phosphorus pentoxide equivalents/m<sup>3</sup> (8,599 mg orthophosphoric acid equivalents/m<sup>3</sup>) for goats (White and Armstrong 1935). Similar lethal concentrations (1,742 mg orthophosphoric acid equivalents/m<sup>3</sup>) were observed in rats exposed to white phosphorus smoke 15 minutes/day, 5 days/week, for 6-13 weeks (Brown et al. 1981; Starke et al. 1982). For the most part, the cause of death was not determined. An exception is the mouse acute exposure study. A thick mucous discharge was observed in the nares of dying mice. This discharge plugged the nares, and the mice died of asphyxiation (White and Armstrong 1935). For the other species tested, the most prominent nonlethal effect was moderate-to-severe respiratory tract irritation. It is possible that the respiratory tract damage was severe enough to be life threatening.*

*Based on this information on deaths in animals, it is likely that exposure to high concentrations of white phosphorus smoke would be fatal to humans.*

### *Respiratory Effects*

*Respiratory tract irritation has been observed in humans exposed to white phosphorus smoke for 2-15 minutes. Throat irritation during talking, coughing, nose irritation, and erythema and edema of the larynx and vocal cords have been reported (Walker et al. 1947; White and Armstrong 1935).*

*Respiratory tract irritation has been observed at concentrations of 187 mg phosphorus pentoxide equivalents/m<sup>3</sup> (258 mg orthophosphoric acid equivalents/m<sup>3</sup>) for 5 minutes or longer (White and Armstrong 1935). Damage to the respiratory tract has also been observed in animals exposed to white phosphorus smoke for acute and intermediate durations. Slight-to-intense congestion, edema, and hemorrhages were observed in the lungs of rats, mice, and goats dying during or following a 1-hour exposure to concentrations of 1,350,470, and 3,870 mg phosphorus pentoxide equivalents/m<sup>3</sup>, respectively (1,863,649, and 5,342 mg orthophosphoric acid equivalents/m<sup>3</sup>) (White and Armstrong 1935) and rats exposed to 3,027 mg orthophosphoric acid equivalents/m<sup>3</sup> for 90 minutes (Brown et al. 1980). Exposure to 1,742 mg orthophosphoric acid equivalents/m<sup>3</sup>*

15 minutes/day, 5 days/week, for 13 weeks resulted in minimal-to-severe interstitial pneumonia in rats (Brown et al. 1981). In addition to these effects in the lungs, slight tracheitis and laryngitis has been observed in rats exposed to 884 mg orthophosphoric acid equivalents/m<sup>3</sup> for 15 minutes/day, 5 days/week, for 6-13 weeks. The severity of these effects on the trachea and larynx increased at higher concentrations (Brown et al. 1981). Dermal studies examining the respiratory tract were not located. Because the respiratory tract effects observed following inhalation exposure to white phosphorus smoke are probably the result of direct contact with the respiratory tissue, it is not likely that similar respiratory tract effects would be observed following dermal exposure.

### *Ocular Effects*

There is limited information on the potential of white phosphorus smoke to induce ocular effects in humans. No human exposure studies examining ocular endpoints were located. In rats, no histological damage in the eye was observed following exposure to 1,742 mg orthophosphoric acid equivalents per cubic metre 15 minutes/day, 5 days/week, for 13 weeks (Brown et al 1981).

### *Hepatic Effects*

There is limited information on hepatotoxicity following exposure to white phosphorus smoke. No human exposure studies examining hepatic end points were identified. In animals, cloudy swelling of the liver was observed following 60-90-minute exposures to 1,170 mg phosphorus pentoxide equivalents/m<sup>3</sup> (1,615 mg orthophosphoric acid equivalents/m<sup>3</sup>) (White and Armstrong 1935) or 3,027 mg orthophosphoric acid equivalents/m<sup>3</sup> in rats (Brown et al. 1980), 470 mg phosphorus pentoxide equivalents/m<sup>3</sup> (649 mg orthophosphoric acid equivalents/m<sup>3</sup>) in mice (White and Armstrong 1935), and 7,320 mg phosphorus pentoxide equivalents/m<sup>3</sup> (10,104 mg orthophosphoric acid equivalents/m<sup>3</sup>) in goats (White and Armstrong 1935). Hepatic effects were not observed in rats exposed to 1,742 mg orthophosphoric acid/m<sup>3</sup> 15 minutes/day, 5 days/week for 6 or 13 weeks (Brown et al. 1981). No studies examining hepatic end points following dermal exposure were located.

### *Renal Effects*

Renal effects were not examined in the three acute-duration white phosphorus smoke inhalation human studies (Walker et al. 1947; White and Armstrong 1935). Slight cloudy swelling in the kidneys was observed in rats, mice, and goats exposed to white phosphorus smoke for 1 hour at concentrations of 1,170, 470, and 7,320 mg phosphorus pentoxide equivalents/m<sup>3</sup>, respectively (1,615, 649, and 10,104 mg orthophosphoric acid equivalents/m<sup>3</sup>) (White and Armstrong 1935). No renal lesions were observed in rats exposed to 3,027 mg orthophosphoric acid/m<sup>3</sup> for 90 minutes (Brown et al. 1980), or rats exposed to 1,742 mg orthophosphoric acid equivalents/m<sup>3</sup> 15 minutes/day, 5 days/week, for 6 or 13 weeks (Brown et al. 1981). Because of differences in the exposure protocols, comparisons between the White and Armstrong (1935) study and the Brown et al. (1980, 1981) studies cannot be made. No dermal exposure studies examining renal effects were located.

### *Reproductive Effects*

*Reproductive end points were not examined in the available human exposure studies (Walker et al. 1947; White and Armstrong 1935). No histological damage was observed in reproductive tissues of male and female rats exposed to 1,742 mg orthophosphoric acid equivalents/m<sup>3</sup> 15 minutes/day, 5 days/week, for 13 weeks (Brown et al. 1981). In addition, no effects on reproductive performance were observed in male or female rats exposed to 1,742 mg orthophosphoric acid equivalents/m<sup>3</sup> for 15 minutes/day, 5 days/week, for 3-13 weeks (Brown et al. 1981; Starke et al. 1982). Dermal exposure studies examining reproductive end points were not located.*

### *Developmental Effects*

*No data on developmental effects in humans exposed to white phosphorus smoke were located. No developmental effects were observed in rats exposed in utero to concentrations of white phosphorus smoke as high as 1,742 mg orthophosphoric acid equivalents/m<sup>3</sup> for 15 minutes/day (Brown et al. 1981; Starke et al. 1982). However, exposure of the dams and pups to white phosphorus smoke in utero and during lactation resulted in an 8% decrease in pup body weight, a 68% decrease in pup survival, and a 35% decrease in viability (Brown et al. 1981; Starke et al. 1982). These results may be due to interference with the pups suckling, decreased milk production, decreased suckling due to respiratory tract irritation in the pups, or another compound-related effect. No dermal exposure developmental studies were located.*

### *Genotoxic Effects and Cancer*

*No studies were located regarding in vivo or in vitro genotoxic effects in humans or animals after inhalation and dermal exposure.*

*No human or animal exposure studies examining cancer following inhalation or dermal exposure to white phosphorus smoke were located.*

The Tenth Report on Carcinogens, by the US National Toxicology Program, did not list white phosphorus or white phosphorus smoke as an agent known to be a human carcinogen or reasonably anticipated to be a human carcinogen.<sup>76</sup>

Review of the database of the International Agency for Research on Cancer revealed no entry regarding an evaluation of the carcinogenicity of white phosphorus or white phosphorus smoke.

Cullumbine (1957) [Chemical Defence Experimental Establishment, Porton, UK] reviewed the toxicity of screening smokes.<sup>77</sup> White phosphorus had been used to produce screening smokes and also as an incendiary agent. The author noted that

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<sup>76</sup> US National Toxicology Program. Tenth Report on Carcinogens. US Department of Health and Human Services. Accessed at <http://ehp.niehs.nih.gov/roc/toc10.html> on 16<sup>th</sup> July 2003

<sup>77</sup> Cullumbine H (1957). The toxicity of screening smokes. Journal of Royal Army Medical Corps 103: 119-122. ID 27841.



white phosphorus smoke produced irritation of throat and coughing. Experiments had shown that a concentration of 700 mg per cubic metre was required before the effects compelled men doing moderate work to adjust their respirators. A concentration of 1,770 mg per cubic metre was needed to harass men similarly at rest. Personnel may be burnt when ignited lumps of phosphorus fall on them.

White phosphorus smoke was recently reviewed (2002).<sup>78</sup> White phosphorus particles burnt fiercely in air, which produced a dense white smoke. Fragments of melted particles of the burning substance may become embedded in the skin of personnel close to a bursting projectile. Burns were multiple, deep and variable in size. White phosphorus smoke is composed of particles of phosphorus pentoxide which are converted by moist air to droplets of phosphoric acid. White phosphorus smoke irritated the eyes and nose in moderate concentrations. Field concentrations of white phosphorus smoke were usually harmless, although they may cause temporary irritation to the eyes, nose or throat. The respirator was considered to provide adequate protection against white phosphorus smoke.

Cataldo et al (1990) assessed the environmental fate and effects of mixed obscurant smokes comprised of white phosphorus, fog oil, and hexachloroethane smokes.<sup>79</sup> The authors noted that there may be potential interaction of HC-derived Cl- with the polyphosphates from WP combustion, and this may influence the fate and effects of these mixed smokes.

Davis (2002) discussed the acute management of white phosphorus burn.<sup>80</sup> These result from the ignition of or burning white phosphorus particles (as opposed to white phosphorus smoke).

The US Occupational Safety and Health Administration has recommended a permissible exposure limit of 0.1 mg per cubic meter for white phosphorus particles.<sup>81</sup> The National Institute for Occupational Safety and Health (NIOSH) considers a concentration of white phosphorus particles of 5 mg per cubic meter immediately dangerous to life or health. NIOSH notes that inhalation of the vapour of white phosphorus particles may cause lung oedema.<sup>82</sup>

### **Case Reports / Series**

Mozingo et al (1988) presented a case series of chemical burns admitted to the US Army Institute of Surgical Research during a 17-year period (1969-1985).<sup>83</sup> 87 of 4,212 burned patients sustained chemical burns. White phosphorus (WP) was the

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<sup>78</sup> Anon (2002). Smokes, fuels, and incendiary materials. J R Army Med Corps 148: 395-7. ID 28186.

<sup>79</sup> Cataldo D, Van Voris P, Ligothe M, et al (1990). Evaluate and characterize mechanisms controlling transport, fate, and effects of army smokes in the aerosol wind tunnel. (Abstract) AD-A221. Pacific Northwest Labs, Richland, WA

<sup>80</sup> Davis K (2002). Acute management of white phosphorus burn. Military Medicine 167: 83-84. ID 27835.

<sup>81</sup> Occupational Safety and Health Administration. US Department of Labor. Accessed at [www.osha.gov](http://www.osha.gov) on 17<sup>th</sup> June 2003. ID 27903

<sup>82</sup> National Institute for Occupational Safety and Health. Pocket Guide to Chemical Hazards / International Chemical Safety Cards. Accessed at [www.cdc.gov/niosh](http://www.cdc.gov/niosh) on 17<sup>th</sup> June 2003. ID 27903

<sup>83</sup> Mozingo D, Smith A, McManus W, et al (1988). Chemical burns. The Journal of Trauma 28: 642-647. ID 28184

most common causative agent; it produced cutaneous injury in 49 patients. Most of these injuries with WP occurred in Vietnam and the clinical records did not always address the presence of WP in the wounds. Systemic toxicity from cutaneous absorption of copper sulfate used to neutralise WP burns occurred in two patients. The average total burn size in WP burns was 22% and the average size of third degree burns was 11.2% in WP burns. WP burns had a mortality of 4.1%. Inhalation injury occurred in two WP burn patients. There was associated eye injury in two WP burn patients but it was unclear whether this was from penetrating trauma or from direct contact of WP particles with the eye. There was no mention of whether any of the burns resulted from exposure to WP smoke, in contrast to, burning WP particles.

## **ADVERSE HEALTH EFFECTS FROM COLOURED SMOKES IN M18 HAND GRENADES**

### **General Reviews / Comments**

The US National Research Council produced a report on the toxicity of military smokes and obscurants.<sup>84</sup> The third volume of this report dealt with coloured smokes in M18 grenades. It found no human studies on old smoke formulations [yellow smoke, green smoke, red smoke, violet smoke], new smoke formulations [yellow smoke, green smoke, red smoke] or its combustion products. However, some concern was expressed over the safety of some of the individual dyes that were components of these smoke formulations. Namely:

Benzanthrone - dermal toxicity in humans [photosensitisation / dermatitis]

Solvent Yellow 33 - allergic contact dermatitis in humans

Disperse Red 9 - dermal irritant / sensitiser in humans

Solvent Red 1 - contact hypersensitivity in animals but the evidence was conflicting

Sections of the Report are reproduced as follows:

*A variety of smokes and obscurants have been developed and used to screen armed forces from view, signal friendly forces, and mark positions. Smokes are produced by burning or vaporizing particular products. Obscurants are anthropogenic or naturally occurring particles suspended in the air. They block or weaken transmission of particular parts of the electromagnetic spectrum, such as visible and infrared radiation or microwaves. Fog, mist, and dust are examples of natural obscurants. White phosphorus and hexachloroethane smokes are examples of anthropogenic obscurants.*

*This volume, Volume 3, reviews the potential toxicity of seven colored smokes used for signaling, marking, and, in some cases, simulating exposure to chemical-warfare agents in military training.*

### **SUBSTANCES EVALUATED**

*Colored smokes are generated by deploying an M18 grenade or 40-mm cartridge containing a pyrotechnic mixture of fuel and dye. The dye mixtures originally formulated by the Army were yellow, green, red, and violet. Because of the potential health hazards of the smoke formulations and the combustion products, the Army developed new formulations for the same colors. However, grenades and cartridges containing the old smoke formulations are still in inventory. Therefore, the Army requested that the NRC evaluate the toxicity of both the old and the new smoke formulations, with the exception of the new violet-smoke formulation, which was removed from the inventory due to its acute toxicity.*

*Using the NRC guidelines published in 1986 and 1992 for recommending exposure guidance levels, the subcommittee evaluated the toxicity data on each of the old*

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<sup>84</sup> National Research Council (2000). Toxicity of Military Smokes and Obscurants. Volume 3. Subcommittee on Military Smokes and Obscurants. National Academy Press: Washington, DC. Accessed at <http://www.nap.edu/openbook/0309065992/html>

and new smoke formulations, the combustion products (smoke), and the individual dye components. The old smoke formulations are listed below with their dye components:

yellow smoke: vat yellow 4 and benzanthrone

green smoke: vat yellow 4, benzanthrone, and solvent green 3

red smoke: disperse red 9

violet smoke: disperse red 9 and 1,4-diamino-2,3-dihydroanthraquinone.

The new smoke formulations are listed below with their dye components:

yellow smoke: solvent yellow 33

green smoke: solvent yellow 33 and solvent green 3

red smoke: solvent red 1 and disperse red 11.

TABLE 1-1 Components of the M18 Colored-Smoke Grenades and 40-mm Colored-Smoke Cartridges (expressed as weight percent)

Component	Yellow				Red				Green				Violet
	M18		40 mm		M18		40 mm		M18		40 mm		M18
	Old	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old
Vat yellow 4	14.0		17.0						4.0		6.0		
Solvent yellow 33		42.0		42.0						12.6		12.5	
Disperse red 9					40.0		44.0						8.4
Solvent red 1						34.2		38.0					
Disperse red 11						6.8		7.0					
Solvent green 3									28.0	29.4	27.3	29.5	
Benzanthrone	24.5		24.0						8.0		11.0		
1,4-Diamino-2,3-dihydroanthraquinone													33.6
Sulfur	8.5		11.2		9.0		9.9		10.4		11.2		9.0
Sodium biocarbonate	33.0		14.7		25.0		17.7		22.6		14.5		24.0
Potassium chlorate	20.0	24.1	28.5	28.4	26.0	17.7	27.9	21.5	27.0	25.0	28.5	28.5	25.0
Magnesium carbonate		17.5		10.3		9.6		4.0		15.5		10.5	
Terephthalic acid						14.0		8.0					
Sugar		16.4		19.3		17.7		21.5		17.5		19.0	
Polyvinyl alcohol				2.0				2.0				2.0	
Stearic acid				0.5		0.5		0.5				0.5	

Source: Adapted from U.S. Army Technical Data Package (1989).

## OLD SMOKE FORMULATIONS

No studies have been conducted in animals or humans on the toxicity of the old yellow-smoke formulation or its combustion products. In one study, rats, mice, and guinea pigs were exposed to a smoke containing benzanthrone, one of the old yellow-smoke components, and a blue dye not present in the Army's old yellow-smoke formulation. Animals exposed at concentrations of 0.9 to 13.4 grams per cubic meter (g/m<sup>3</sup>) for 1 hr exhibited injury characterized by lung necrosis, sloughing of the mucosa, edema in the alveolar space, and necrosis in the tracheobronchial tree.

*No studies have been conducted on the toxicity of the old green-smoke formulation or its combustion products. In two studies rats, mice, and guinea pigs were exposed to smokes containing solvent green 3, one of the dye components of the old formulation. The smokes used in those studies also contained dye components not present in the Army's old green smoke. In one study, animals exposed to smoke containing solvent green 3 and auramine hydrochloride at concentrations of 0.6 to 12.1 g/m<sup>3</sup> for 1 hr exhibited injury characterized by lung necrosis, sloughing of the mucosa, and edema in the alveolar space. In the other study, animals were exposed to a smoke composed of solvent yellow 33, disperse red 9, and solvent green 3 at concentrations of 0.1 to 1.0 g/m<sup>3</sup> for 1 hr per day, 5 days per week for 100 days. The dye was retained in the lungs of all animals. Histological examination revealed pulmonary congestion, alveolitis, chronic pneumonia, and lung inflammation.*

*Old red smoke was evaluated for acute toxic effects in several animal species. Rats, rabbits, guinea pigs, dogs, swine, and goats were exposed to old red smoke at concentrations of 1.5 to 18 g/m<sup>3</sup> for 10 to 240 min. All animals showed signs of upper-respiratory-tract irritation and salivation immediately after exposure, and all had labored breathing for 7 days after the exposure. The study reported the combined mortality of the total number of animals of all the species exposed to the smoke. Only general information can be obtained from this study, because the mortality results in individual species were not given.*

*Slaga et al (1985) examined the initiation and promotion properties of the old red-smoke formulation in the Sencar mouse. The smoke formulation was studied for its complete carcinogenicity (administered as an initiator and a promoter to the same animals) or as an initiator only (administered as an initiator followed by 12-O-tetradecanoyl phorbol 13-acetate administration). There was no tumor response when the smoke formulation was tested as a complete carcinogen or as an initiator.*

*The toxicity of old violet smoke was evaluated in a study using rats, rabbits, guinea pigs, dogs, swine, and goats. Animals were exposed at concentrations of 1.3 to 7.8 g/m<sup>3</sup> for 8 to 142 min. All animals showed upper-respiratory-tract irritation and salivation. As in the study of old red smoke, only general information can be obtained from this study because the results were reported as the combined mortality of the total number of animals of all the species exposed to the smoke. The old violet-smoke formulation was tested for mutagenicity and found to be positive in the Ames assay.*

*Slaga et al (1985) conducted studies of the ability of the old violet-smoke formulation to exhibit complete carcinogenicity as well as its ability to be an initiator in the Sencar mouse. There was no tumor response to the formulation when tested as a complete carcinogen or as an initiator.*

#### **NEW SMOKE FORMULATIONS**

*No studies have been conducted in animals or humans on the toxicity of the new yellow-smoke formulation or its combustion products. However, two studies evaluated the toxicity of smokes containing solvent yellow 33, the major*

*component of the new yellow-smoke formulation. The smokes used in both studies also contained dyes not present in the Army's new formulation. One study used a smoke containing solvent yellow 33, solvent green 3, and disperse red 9. In that study, histological examination of rats, mice, and guinea pigs exposed to the smoke at concentrations of 0.1 to 1.0 g/m<sup>3</sup> for 1 hr per day, 5 days per week for 100 days revealed pulmonary congestion, alveolitis, chronic pneumonia, and lung inflammation. The other study evaluated the toxicity of a smoke containing solvent yellow 33 and disperse orange 11. Mice, rats, and guinea pigs were exposed at 0.11 to 1.0 g/m<sup>3</sup> for 1 hr per day, 5 days per week for 200 days. Toxic effects appeared to be confined to the respiratory tract. Lymphocyte infiltration in the larynx and trachea of mice and guinea pigs and dilated mucous glands in the trachea of mice and rats were reported.*

*The only data on the toxicity of the new green-smoke formulation are from an inhalation study on a mixture of solvent yellow 33 and solvent green 3 aerosols. The mixture was not acutely toxic; however, mild pulmonary inflammation was observed and was attributed to solvent green 3. No-observed-adverse-effect levels for the aerosolized mixture were 50 milligrams (mg)/m<sup>3</sup> for a 4-week exposure and 10 mg/m<sup>3</sup> for a 13-week exposure. Two other studies might have some relevance in assessing the potential toxicity of new green smoke, although the smoke formulation did not have the same composition as the Army's. One study evaluated the toxicity of a smoke containing solvent yellow 33, solvent green 3, and disperse red 9. Histological examination of rats, mice, and guinea pigs exposed to the smoke at concentrations of 0.1 to 1.0 g/m<sup>3</sup> for 1 hr per day, 5 days per week for 100 days revealed pulmonary congestion, alveolitis, chronic pneumonia, and lung inflammation. Another study evaluated the toxicity of a smoke composed of solvent yellow 33 and disperse orange 11. Mice, rats, and guinea pigs were exposed at 0.11 to 1.0 g/m<sup>3</sup> for 1 hr per day, 5 days per week for 200 days. Toxic effects appeared to be confined to the respiratory tract; lymphocyte infiltration in the larynx and trachea of mice and guinea pigs and dilated mucous glands in the trachea of mice and rats were reported.*

*No studies have been conducted using new red smoke. Data are available on the toxicity of an aerosolized mixture containing solvent red 1 and disperse red 11. Inhalation exposure of rats and rabbits to the aerosolized mixture resulted in nasal and lung lesions. Only minimal details of the study are available; therefore, the study's scientific merits cannot be evaluated adequately, and the information cannot be used to recommend exposure guidance levels.*

#### **INDIVIDUAL DYE COMPONENTS**

*In addition to evaluating the toxicity data on the smoke formulations and the combustion products, the subcommittee reviewed toxicity data on the individual dyes used in the smoke formulations. Toxicity data for nine dyes were reviewed and summarized. Concern about the toxicity of several of them is substantial. For example, four dyes that have been demonstrated to be dermal sensitizers in humans and laboratory animals are benzanthrone, a component of the old yellow- and old green-smoke formulations; solvent yellow 33, a component of the new yellow- and new green-smoke formulations; solvent red 1, a component of the new red-smoke formulation; and disperse red 9, a component of the old red- and violet-smoke*

*formulations. Additionally, their potential for pulmonary sensitization after inhalation of the smokes has not been addressed adequately and remains a source of uncertainty. Because each of the seven smokes evaluated contains one of the four dyes identified as sensitizing agents, that uncertainty applies to all smokes under consideration.*

## CONCLUSIONS AND RECOMMENDATIONS

*The subcommittee concludes that the available toxicity data base for the combustion products of the old and new smoke formulations is inadequate for use in assessing the potential health risk of exposure to these smokes and in recommending exposure guidance levels. Review of the data identified sufficient evidence of toxicity for smoke formulations and combustion products to raise concern, particularly with regard to dermal and respiratory-tract sensitization. However, the data are too sparse to permit well-informed recommendations for exposure guidance levels. Stringent guidance levels could be arbitrarily set to protect personnel; however, the Army's current policy states that troops without protective clothing must avoid entering the smoke cloud during training. That policy should serve to protect troops until further research can be performed to provide more information for recommending exposure guidance levels.*

*The primary reason for the subcommittee's concern about the potential toxicity of the colored smokes is the demonstration of contact allergic dermatitis in humans and laboratory animals exposed to several of the dyes that are components of the old and new smokes.*

Dacre (1994) reviewed almost 20 years of research on Army chemicals conducted in the US Army Biomedical Research and Development Laboratory at Fort Detrick, Maryland.<sup>85</sup> Compounds said to contribute to the production of coloured smokes were benzanthrone, graphite, solvent yellow 33, disperse red 9, solvent violet 47 and solvent green 3. The author noted that the azo dye components have been found to be carcinogenic in mammalian toxicity studies. The specific azo dyes implicated were not mentioned.

### **Benzanthrone**

Volume 3 of the US National Research Council Report (2000) on the toxicity of military smokes and obscurants dealt with coloured smokes in M18 grenades.<sup>86</sup> This report reviewed data on benzanthrone (BZA). Relevant sections are reproduced as follows.

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<sup>85</sup> Dacre J (1994). Hazard evaluation of Army compounds in the environment. *Drug Metabolism Reviews* 26: 649-662. ID 28306

<sup>86</sup> National Research Council (2000). *Toxicity of Military Smokes and Obscurants. Volume 3.* Subcommittee on Military Smokes and Obscurants. National Academy Press: Washington, DC. Accessed at <http://www.nap.edu/openbook/0309065992/html>

### *Effects in Humans*

*BZA is reported to cause an itching and burning sensation, erythema, dermatitis, and skin pigmentation (Uebelin and Buess 1951; Singh and Zaidi 1969; Trivedi and Niyogi 1968; Schwartz et al. 1957; Schwartz 1939; Horakova and Merhaut 1966, as cited in Dacre et al.1979). In sensitive individuals, actinic dermatitis or leukoderma can develop because of a photodynamic effect (Singh and Zaidi 1969; Trivedi and Niyogi 1968; Schwartz et al. 1957; Schwartz 1939; Horakova and Merhaut 1966; Isaev et al.1957; Hueper 1942, as cited in Dacre et al.1979). Itching, precocious generalized eczema, pigmentation, and photosensitization have been observed in workers exposed to BZA (Horakova and Merhaut 1966). Systemic effects result from liver damage (Slutskii 1958), nervous-system damage (Piskunova et al.1956), and disturbance of the autonomic-nervous-system regulatory function (Slutskii 1958). More recent studies indicate that BZA, upon exposure to light, can generate active oxygen species that might be responsible for the photo-contact dermatitis caused by BZA in industrial workers exposed to this chemical (Dabestani et al.1992). Because of the toxic nature of BZA, the U.S. Army Environmental Health Agency advised substituting BZA with a less toxic chemical in smoke mixtures (AEHA 1970).*

### *Effects in Animals*

*Initial studies do not indicate that BZA is carcinogenic in mice (Parent 1964). Tests for mutagenicity in the dominant lethal mouse assay were negative (Epstein et al 1972). BZA was not mutagenic in Salmonella typhimurium or Escherichia coli strains in some reports (Brown and Brown 1976; Gibson et al 1978; Bond and Gilleland 1955), but Epler (1979) reported BZA to be mutagenic in the Salmonella assay.*

Singh et al (1990) reviewed recent studies on the toxicity of benzanthrone (BZA).<sup>87</sup> BZA is a dye intermediate used in the manufacture of several polycyclic vat and disperse dyes. Skin Effects: Piskunova et al (1956) [in Russian] observed dermatitis and dark skin colouration in many workers with exposure to BZA. Horakova and Merhaut (1966) [in Polish] observed pruritis to generalised eczema, rarely pigmentation and photosensitisation, in 107 workers engaged in the manufacture of BZA for a period of four years. Trivedi and Niyogi (1968) clinically examined 48 workers in a BZA-producing dye factory. An air sample contained 0.63 ug of BZA per cubic meter. 30% of workers had skin blackening and 14% had only a burning sensation. Keratosis was observed in two subjects. Skin blackening had occurred with one to 35 months of exposure. Singh and Zaidi (1969) conducted extensive clinical examinations on 25 cases from the dye factory. Subjects were males aged 22 to 60 years. Toxic symptoms developed in the majority within one to two years. Initially there was skin pigmentation with a burning and itching sensation. Formation of vesicles occurred in 20% of cases, with marginal redness and pigmentation upon rupture. In another 20% of cases, pigmentation affected the entire body. In the remaining 60%, pigmented patches associated with itching, roughness and dryness affected only exposed areas of the body. Exposure to sunlight exacerbated the burning and itching sensation in pigmented areas. Skin disorders, including photodermatitis,

<sup>87</sup> Singh G, Khanna S, Das M (1990). Recent studies on toxicity of benzanthrone. Journal of Scientific and Industrial Research 49: 288-296. ID 28302



were also observed in 60 workers with occupational exposure to BZA in a manufacturing unit, where higher than permissible concentration of BZA was detected (Kleiner et al 1979, Timoshenko et al 1980 - reports from the USSR).

A range of effects on other systems in humans was also reported.<sup>88</sup> An increased frequency of cold, cough and bronchitis (Trivedi and Niyogi 1968, Singh and Zaidi 1969) and disorders of the upper respiratory tract (Kleiner et al 1979) were reported in several workers employed in the manufacture of BZA. Disturbed liver function has been mentioned in reports from the USSR of workers involved in the manufacture of BZA (Kleiner et al 1979, Timoshenko et al 1980) or with BZA intoxication (Slutzkii 1957), but details were scant. Slutzkii (1958) studied 42 patients intoxicated by BZA. Disturbance of the nervous system and autonomic nervous system has also been associated with BZA toxicity in reports from the USSR (Piskunova et al 1956, Kleiner et al 1979, Slutzkii 1957, 1958). Headache, giddiness and depression were also reported by several workmen exposed to BZA during its manufacture in a dye factory (Singh and Zaidi 1969). Increased cardiovascular diseases have also been reported (Timoshenko et al 1980). Several workers exposed to BZA during its manufacture were observed to be anaemic (Trivedi and Niyogi 1968, Singh and Zaidi 1969), although the former study found no significant difference in haemoglobin between BZA affected workers and controls. Workers employed in the production of BZA have also complained of a decrease in sexual activity or low fertility (Horakova and Merhaut 1966, Singh and Zaidi 1969). Singh and Zaidi (1969) also reported that several of their subjects complained of loss of appetite, general weakness, disturbed sleep and within three to six months of exposure, a slight decrease in weight. The latter was ascribed to the strain of disturbed sleep and psychological affects from skin pigmentation.

Animal studies.<sup>89</sup> Singh et al (1967) observed skin thickening and roughness after single topical application of BZA to mice followed by one-hour exposure to sunlight for 20 and 40 days. Histology of BZA-photosensitised skin after 20 days, showed oedematous dermatitis, after 40 days, there was skin thickening with hyperkeratinisation. The cells in the stratum spinosum were hyperplastic and at places showed hyperchromasia. The dermis showed a marked increase of fibrous tissue and infiltration with mononuclear cells. Only mild histological changes were seen in animals kept in the dark following BZA treatment. The author suggested that sunlight potentiated the toxic action of BZA on the skin. The mechanism of action in BZA-induced skin lesions was also thought to involve ascorbic acid. Lung parenchyma was filled with oedema and haemorrhage in guinea pigs administered BZA intratracheally, within 24 hours (Singh 1971). After 48 hours, phagocytic cells had increased and changes persisted in lungs for 144 hours. After 15 days, lungs were found to be normal. Histological studies of testis in BZA-treated animals for a period of 6 months showed patchy degeneration involving 30-40% of seminiferous tubules (Singh and Khanna 1975). Administration of BZA in rats was associated with normocytic anaemia (Chandra and Singh 1968).

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<sup>88</sup> Singh G, Khanna S, Das M (1990). Recent studies on toxicity of benzanthrone. *Journal of Scientific and Industrial Research* 49: 288-296. ID 28302

<sup>89</sup> Singh G, Khanna S, Das M (1990). Recent studies on toxicity of benzanthrone. *Journal of Scientific and Industrial Research* 49: 288-296. ID 28302

Mutagenicity studies.<sup>90</sup> BZA was not mutagenic when tested with five strains of *Salmonella typhimurium*, with and without mammalian metabolic activation (Brown and Brown 1976). After exposure to  $\gamma$ -irradiation in air, BZA was mutagenic with some strains of *S. typhimurium* (Gibson et al 1978). BZA was not mutagenic in *E. coli*, i.e. did not alter the RNA/DNA ratio (Bond and Gilleland 1955). Epstein et al (1972) conducted a dominant lethal mouse assay for BZA-induced mutations. Male Swiss mice were given intraperitoneal injections of BZA, at doses of 1000, 1500 or 2000 mg/kg. Surviving males were mated to untreated female mice for two to eight weeks after the injection. There was no significant difference in early fetal deaths and pre-implantation losses in BZA-treated mice compared to controls.

Carcinogenicity studies.<sup>91</sup> White mice were given five subcutaneous injections, 10 to 15 days apart, of 0.5% BZA in olive oil. In 16 of 32 mice that survived after 6 months, one developed a lung tumour and one developed a jaw tumour (Morosenskaya 1940 - USSR, Hartwell 1951). These studies were interpreted as BZA having no specific tumourigenic properties. BZA (0.3 g in solution) was applied to the interscapular region of mice twice weekly. No skin epitheliomas or papillomas were observed in 10 mice treated for 294 days (Barry et al 1935). Parent (1964) reported that BZA did not induce tumours in mice and Morosenskaya (1940) reported no tumours after painting or injecting mice with large doses of BZA.

Benzanthrone is manufactured by heating the reduction product of anthraquinone with sulfuric acid and glycerol.<sup>92</sup> BZA is referred to as an anthraquinone-dye intermediate. Exposure to benzanthrone can occur through dermal contact, inhalation and ingestion. Persons with exposure between months and years complain of loss of appetite, intolerance of fatty foods, fatigue, weakness, decreased sexual potency, and weight loss. There have been reports of neurasthenic syndrome, changes in corneal and cremaster reflexes, accelerated pulse and reduced blood pressure, impairment of liver function and gastritis with decreased acidity (Encyclopedia of Occupational Health and Safety, volumes 1 and 2, page 166-167, 1971).

The CCRIS database identified a study by Moeller et al (1985) that examined the mutagenicity of polycyclic aromatic hydrocarbons in source emissions and ambient air.<sup>93</sup> The Ames *Salmonella typhimurium* test system was used. BZA was negative in strain TA 98 without metabolic activation. But it was positive in strains TA 98 and TA 100 with metabolic activation (rat liver S9, aroclor 1254).

Durant et al (1996) evaluated 67 polycyclic aromatic compounds thought to be present in urban air for mutagenicity via a forward mutation assay based on human B-lymphoblastoid cells.<sup>94</sup> Mutagenicity was measured at the thymidine kinase locus in the human B-lymphoblastoid cell line designated h1A1v2. Cultures were grown to allow for the phenotypic expression of mutations. Benzanthrone [7H-

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<sup>90</sup> Singh G, Khanna S, Das M (1990). Recent studies on toxicity of benzanthrone. *Journal of Scientific and Industrial Research* 49: 288-296. ID 28302

<sup>91</sup> Singh G, Khanna S, Das M (1990). Recent studies on toxicity of benzanthrone. *Journal of Scientific and Industrial Research* 49: 288-296. ID 28302

<sup>92</sup> Accessed at <http://toxnet.nlm.nih.gov> On 21<sup>st</sup> July 2003.

<sup>93</sup> CCRIS. Accessed at <http://toxnet.nlm.nih.gov> On 21<sup>st</sup> July 2003

<sup>94</sup> Durant J, Busby W, Lafleur A, et al (1996). Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. *Mutat Res* 371: 123-57. ID28595

benz(de)anthracen-7-one] was tested at five doses (10 to 10,000 ng/ml) and cells exposed to it for 72 hours in duplicate cultures with positive (benzo[a]pyrene) and negative (dimethyl sulfoxide) controls. Benzanthrone was observed to be mutagenic in this assay, but it was less active than a standard, benzo[a]pyrene. The minimum mutagenic concentration of benzanthrone was approximately 250 fold higher than that of benzo[a]pyrene. The authors concluded that benzanthrone was not an important mutagen. The authors noted the results of another in-vitro study that tested the constituents of kerosene soot for mutagenicity in bacteria (Kaden et al 1979). This latter study found that benzanthrone was mutagenic with a minimum mutagenic concentration only five fold higher than that of benzo[a]pyrene.

#### **Vat Yellow 4**

IARC (1990) concluded that vat yellow 4 was not classifiable as to its carcinogenicity to humans (Group 3), based on limited evidence for carcinogenicity in experimental animals and no data were available from studies in humans.<sup>95</sup>

*Chem. Abstr. Name: Dibenzo[b,def]chrysene-7,14-dione*

*Vat Yellow 4 is an anthraquinone-type dyestuff which is used to colour fabrics and paper and in smoke-screen formulations for military use. No data on occupational exposure levels were available.*

##### *5.2 Experimental carcinogenicity data*

*Vat Yellow 4 was tested for carcinogenicity by oral administration in one strain of mice and in one strain of rats, producing an increased incidence of lymphomas and hepatocellular tumours in male mice.*

##### *5.3 Human carcinogenicity data*

*No data were available to the Working Group.*

##### *5.4 Other relevant data*

*In a single study, Vat Yellow 4 was not mutagenic to bacteria in the presence or absence of an exogenous metabolic system.*

##### *5.5 Evaluation*

*There is limited evidence for the carcinogenicity of Vat Yellow 4 in experimental animals.*

*No data were available from studies in humans on the carcinogenicity of Vat Yellow 4.*

##### *Overall evaluation*

*Vat Yellow 4 is not classifiable as to its carcinogenicity to humans (Group 3).*

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<sup>95</sup> International Agency for Research on Cancer (1990). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 48: Some Flame Retardants and Textile Chemicals, and Exposures in the Textile Manufacturing Industry. Lyon: France.

## **Disperse Red 9**

Volume 3 of the US National Research Council Report (2000) on the toxicity of military smokes and obscurants dealt with coloured smokes in M18 grenades.<sup>96</sup> This report reviewed data on disperse red 9 [1-(methylamino)-9,10-anthracenedione or 1-N-methylamino-9,10-anthraquinone]. Relevant sections are reproduced as follows.

### *Effects in Humans*

*Disperse red 9 is reported to be a skin irritant and sensitizer in humans (Dacre et al 1979; Owens and Ward 1974). Exposure concentrations were not reported.*

### *Effects in Animals*

*Disperse red 9 has a toxicity rating of 1 which means that it is slightly toxic and its effects are temporary.*

*Studies have used the Ames assay to test disperse red 9 for mutagenicity. Lundy and Eaton (1994) reported a positive response. Dacre et al (1979) cited a study that concluded that there was no evidence of mutagenicity. A review of anthraquinone dyes as candidates for nomination to the National Cancer Institute's Chemical Selection Working Group for carcinogenesis bioassay is described by Sigman et al (1985). Disperse red 9 was considered for study because it gave positive results for mutagenicity in mouse lymphoma cells with and without activation and positive effects in the unscheduled DNA synthesis assay with mouse liver S9. Other mutagenicity tests (ie Ames, dominant lethal, mitotic gene conversion) gave negative results.*

*In a carcinogenicity study by Griswold et al (1968), one kidney tumor was identified in a female rat 9 months after disperse red 9 was administered at 5g per rat (total dose) by gavage. The results produced inadequate evidence of carcinogenicity.*

*The combustion product 2-AA was found to be carcinogenic in bioassays using rats and mice (NCI 1978).*

## **Aminoanthraquinones**

Volume 3 of the US National Research Council Report (2000) on the toxicity of military smokes and obscurants dealt with coloured smokes.<sup>97</sup> In this report it mentioned that the combustion products of 1-methylaminoanthraquinone (the major component of old red smoke and a minor component of old violet smoke) were 1- and

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<sup>96</sup> National Research Council (2000). Toxicity of Military Smokes and Obscurants. Volume 3. Subcommittee on Military Smokes and Obscurants. National Academy Press: Washington, DC. Accessed at <http://www.nap.edu/openbook/0309065992/html>

<sup>97</sup> National Research Council (2000). Toxicity of Military Smokes and Obscurants. Volume 3. Subcommittee on Military Smokes and Obscurants. National Academy Press: Washington, DC. Accessed at <http://www.nap.edu/openbook/0309065992/html>

2-aminoanthraquinones. Some information on 2-aminoanthraquinone was identified as follows.

The US Department of Health's Report on Carcinogens (Tenth Edition) considered that 2-aminoanthraquinone was reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals.<sup>98</sup> When administered in the diet, 2-aminoanthraquinone increased the incidences of hepatocellular carcinomas and neoplastic nodules in male rats, hepatocellular carcinomas in mice of both sexes and lymphomas in female mice. There was no adequate data available in humans.

IARC (1987) assessed 2-aminoanthraquinone as group 3 - not classifiable as to its carcinogenicity to humans.<sup>99</sup> There was no adequate data available in humans and limited evidence for carcinogenicity in animals. The previous assessment (vol 27, 1982) is reproduced<sup>100</sup>.

*2-Aminoanthraquinone (technical grade of low purity) was tested in one experiment in mice and in one experiment in rats by dietary administration. It produced hepatocellular carcinomas in mice of both sexes and in male rats. Purified 2-aminoanthraquinone was not mutagenic to Salmonella typhimurium.*

#### *5.2 Human data*

*2-Aminoanthraquinone has been produced commercially since at least 1921. Its use as an intermediate in the manufacture of dyes and pigments could result in occupational exposure.*

*No case report or epidemiological study was available to the Working Group.*

#### *5.3 Evaluation*

*There is limited evidence for the carcinogenicity in experimental animals of the material tested, which was technical-grade 2-aminoanthraquinone of low purity. In view of the uncertain purity of the compound tested and in the absence of data on humans, no evaluation of the carcinogenicity of 2-aminoanthraquinone could be made.*

## **Anthraquinones**

Bernstein (1997) reviewed occupational asthma.<sup>101</sup> In a table of reactive chemicals that cause occupational asthma was anthraquinone dyes. Brooks (1998) in his review of occupational asthma noted that anthraquinone was an example of an agent that caused occupational asthma through an IgE mechanism.<sup>102</sup>

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<sup>98</sup> US National Toxicology Program. Tenth Report on Carcinogens. US Department of Health and Human Services. Accessed at <http://ehp.niehs.nih.gov/roc/toc/toc10.html> on 16<sup>th</sup> July 2003

<sup>99</sup> IARC (1987). Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. Supplement 7. Lyon, France. D21.1

<sup>100</sup> IARC (1982). Some aromatic amines, anthraquinones and nitroso compounds, and inorganic fluorides used in drinking water and dental preparations. IARC Monograph 27. Lyon, France. Accessed at <http://www-cie.iarc.fr/htdocs/monographs/vol27/2-aminoanthraquinone.html> on 16<sup>th</sup> May 2003

<sup>101</sup> Bernstein D (1997). Chapter 12 - allergic reactions to workplace allergens. JAMA 278: 1907-13. ID 22815.

<sup>102</sup> Brooks S (1998). Occupational and environmental asthma. In: Environmental and Occupational Medicine Third Ed. W. Rom (Ed). Chapter 33, p 487. Lippincott-Raven ID B21

## **ADVERSE HEALTH EFFECTS FROM COLOURED SMOKES IN "L" SERIES SMOKE GRENADES**

### **General Reviews / Comments**

No studies in humans or animals exposed to the coloured formulations of number 83 hand grenades or the combusted smokes were found. No studies in humans or animals exposed to the coloured formulations of "L" series grenades or the combusted smokes were found. Several databases were searched: Medline, OSHA, NIOSH, IARC, US National Toxicology Program, Agency for Toxic Substances and Disease Registry, Toxnet and MSDS.

Some dye components of formulations of "L" series smoke grenades are discussed below. Disperse red 9 was previously discussed in the section on M18 grenades and no studies were found on solvent green 3A.

### **Solvent Yellow 33**

Volume 3 of the US National Research Council Report (2000) on the toxicity of military smokes and obscurants dealt with coloured smokes in M18 grenades.<sup>103</sup> This report reviewed data on solvent yellow 33 since this was one of the dye components in new coloured smoke formulations, as mentioned previously. Solvent yellow 33 is also known as 2-(2-quinolyyl)-1,3-indandione; D&C yellow number 11; quinazoline yellow spirit soluble and yellow number 204. Relevant sections concerning solvent yellow 33 are reproduced as follows:

#### *Smokes Containing Solvent Yellow 33*

*One study used a smoke containing solvent yellow 33, solvent green 3, and disperse red 9 [Marrs et al 1984]. In that study, histological examination of rats, mice, and guinea pigs exposed to the smoke at concentrations of 0.1 to 1.0 g/m<sup>3</sup> for 1 hr per day, 5 days per week for 100 days revealed pulmonary congestion, alveolitis, chronic pneumonia, and lung inflammation. The other study evaluated the toxicity of a smoke containing solvent yellow 33 and disperse orange 11 [Marrs et al 1988]. Mice, rats, and guinea pigs were exposed at 0.11 to 1.0 g/m<sup>3</sup> for 1 hr per day, 5 days per week for 200 days. Toxic effects appeared to be confined to the respiratory tract. Lymphocyte infiltration in the larynx and trachea of mice and guinea pigs and dilated mucous glands in the trachea of mice and rats were reported.*

*Marrs et al (1988) noted that the large number of deaths observed in the high-dose group of guinea pigs was not unprecedented, because a similar phenomenon was observed in a previous inhalation study in which guinea pigs were exposed to the combustion products of a brown smoke-formulation containing a mixture of three dyes: solvent green 3, solvent yellow 33, and disperse red 9 (Marrs et al 1984; reviewed in Chapter 2).*

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<sup>103</sup> National Research Council (2000). Toxicity of Military Smokes and Obscurants. Volume 3. Subcommittee on Military Smokes and Obscurants. National Academy Press: Washington, DC. Accessed at <http://www.nap.edu/openbook/0309065992/html>

Chapter 2). The authors commented that the lethality might be a reflection of the peculiar sensitivity of guinea pigs to the stress associated with inhalation of particles and suggested that the cause of death was due to bronchial spasms. However, solvent yellow 33 was a component of both the brown-smoke and the yellow-orange-smoke formulations. Thus, its role in the hypersensitivity of the guinea pigs to the inhaled combustion products cannot be discounted. In some cases, guinea pigs have been shown to be an appropriate animal model for study of human hypersensitivity to inhaled materials (Mauderly 1984).

Marrs et al. (1988) suggested that the combustion products of the yellow-orange-smoke formulation were clearly toxic to all test groups of the three species studied, because the relative body weights decreased during the exposure period. However, organ-specific toxicity appeared to be confined to the respiratory tract. In the surviving animals, a noteworthy and significant difference from the previously tested combustion products of the brown-smoke formulation was the absence of retained dye in the lungs of any species and the absence of sheets of packed macrophages. Those two observations were previously reported in rats exposed to the combustion products of the brown-smoke formulation containing three dyes (Marrs et al. 1984).

Marrs et al. (1988) concluded that the findings in the respiratory tract of the animals exposed to the combustion products of both the yellow-orange-smoke and the brown-smoke formulations represented the less specific effects of smoke toxicity rather than the effects of the dye components. They suggested that their findings might be common to all military smokes and were probably caused by the nondye constituents. Their findings included lymphocyte infiltration into the larynx and trachea in the mice and guinea pigs and dilated mucous glands in the trachea of mice and rats. The authors concluded that the findings could be explained on the basis of previous studies of the products of reaction between potassium chlorate and lactose, the main components of the resulting reaction being carbon dioxide, water, and potassium chloride (Marrs et al. 1988).

*Toxicokinetics studies by Medinsky et al (1986) found that solvent green 3 is retained in the lungs of animals following inhalation suggesting that the differences in the respiratory effects of the two combustion products might be due to the presence of solvent green 3 in the brown-smoke formulation.*

*Inhalation studies have also been conducted on a mixture of solvent yellow 33 and solvent green 3 aerosols. Sun et al (1987) conducted 4-week and a 13-week inhalation toxicity studies of a 70:30 mixture of solvent green 3 and solvent yellow 33 in rats. The solvent yellow 33 cleared the lungs rapidly, and most of the pulmonary toxicity resulting from the exposures was attributed to the solvent green 3 which remained in the lungs with a calculated half-life of 280 days based on clearance observed during the 30-day post-exposure period. The mixture was not highly toxic, but at the highest exposure concentration (0.2 g per cubic metre for the 4-week exposure and 0.1 g per cubic metre for the 13-week exposure), the rats had a mild pulmonary inflammation, slight type-II cell hyperplasia, and an accumulation of vacuolated alveolar macrophages in the lungs. No other organs were affected. No effects were observed at the two lower exposure concentrations*

(0.01 and 0.05 g per cubic metre for the 4-week exposure and 0.001 and 0.01 g per cubic metre for the 13-week exposure).

### *Dye Solvent Yellow 33*

*The primary dye component of the new yellow-smoke formulation is solvent yellow 33, a demonstrated human contact allergen (reviewed in Appendix C). Although most case reports and controlled studies have focused on dermal contact sensitivity, the sensitizing potential of inhaled solvent yellow 33 cannot be discounted. No studies in the appropriate animal models have been conducted to explore the potential for respiratory-tract sensitization after exposure to inhaled solvent yellow 33 particles.*

### *Effects in Humans*

*There are numerous reports of contact dermatitis in humans exposed to solvent yellow 33. Some of the most recent case reports and clinical studies are presented in Table C-1. Solvent yellow 33 is used in soaps, shampoos, and other externally applied products. Case reports of allergic contact dermatitis due to exposure to solvent yellow 33 in cosmetics, such as eyeliner, rouge, and lipstick have been published (reviewed by Feinman and Doyle 1988). Weaver (1983a) reported that a soap containing solvent yellow 33 at 60 parts per million (ppm) elicited dermatitis in two consumers. Monk (1987) reported allergic contact dermatitis from exposure to solvent yellow 33 found in a hair cream. Reactivity was confirmed using a patch test. There is one report of a worker diagnosed with occupational allergic contact dermatitis (Noster and Hausen 1978). The individual was employed in a factory that manufactured colored smokes for use in detonators.*

*Controlled laboratory studies with human volunteers confirm the sensitizing potential of solvent yellow 33. Jordan (1981) describes contact dermatitis due to solvent yellow 33 in 11 of 149 volunteers who developed a sensitization reaction when tested with solvent yellow 33 concentrations of 16.4 ppm in a modified Draize test. Weaver (1983b) demonstrated sensitization in a repeated-insult patch test of Solvent yellow 33 at concentrations as low as 10 ppm but not at 5 ppm. Rapaport (1984) induced sensitization in 14 of 56 healthy volunteers using a solution containing 20% solvent yellow 33. Bjorkner and Magnusson (1981) and Bjorkner and Niklasson (1983) report sensitization in 4 of 88 normal volunteers exposed to 1% solvent yellow 33 in propylene glycol and in one patient exposed to 0.00001% of the dye. Kita et al. (1984) demonstrated sensitization to solvent yellow 33 in 15 of 20 subjects exposed to 0.5% solvent yellow 33 in petrolatum. All 15 allergic subjects reacted to challenge with solutions containing 1,000 ppm, and one reacted to challenges down to 1 ppm. Kita and colleagues concluded that solvent yellow 33 is a potent contact sensitizer because 15 of 20 subjects became sensitized using the maximization test, and almost half of those individuals reacted to challenge with 100 ppm. The authors also investigated the cross-reactivity to purified samples of D&C yellow no.10. Solutions containing 5% of that dye failed to sensitize human subjects. D&C yellow no.10 is the sodium salt of solvent yellow 33 and is soluble and ionised in aqueous solutions. Weaver (1983b) attributes the differences in the sensitization potential of the two dyes to the ability of solvent*



yellow 33 to penetrate the skin more readily.

*Solvent yellow 33 is a contact allergen in some humans. Thus, even respiratory protection will not be completely effective in protecting sensitive military personnel. The respiratory sensitizing potential of solvent yellow 33 has not been adequately investigated in animals or humans. Additionally, studies testing solvent yellow 33 for mutagenicity are inconclusive.*

**TABLE C-1** Summary of Human Dermal Sensitization Associated with Solvent Yellow 33

Study Population	Exposure Conditions	Response and Comments	Reference
Case report, 38-year-old male	Manufacture of smoke detonators	Contact dermatitis	Noster and Hausen 1978
Clinical study, 56 volunteers	Repeated insult patch test; 5 exposures to 20% solvent yellow 33 in petrolatum, 2 challenges	Sensitization in 15/56 (27%)	Rappaport 1984
Clinical study, 149 volunteers	Modified Draize test	11/149 (7.4%) sensitized at 16.4 ppm	Jordan 1981
Clinical study, 88 volunteers	1% solvent yellow 33 in propylene glycol	4/88 (4.5%) positive patch response at 72 hr	Bjorkner and Magnusson 1981
Case reports, 50-yr-old female, 61-yr-old male	Soap, 0.006% solvent yellow 33	Dermatitis, sensitization, vesicular dermatoses	Weaver 1983a
Clinical study, 2 patients	Repeated-insult patch test; 5, 10, and 20 ppm	Sensitization at 10 and 20 ppm; no sensitization at 5 ppm	Weaver 1983b
Clinical study, 1 patient	Patch test on upper back, various concentrations	Positive patch test to 0.00001%	Bjorkner and Niklasson 1983
Clinical study, 20 volunteers, 18-34 years old	Maximization test, 5 exposures to 0.5% solvent yellow 33 in petrolatum; 1 challenge with 1,000 to 0.1 ppm	15/20 (75%) contact sensitization	Kita et al. 1984
Case report, 50-yr-old male	Hair cream; patch test, 1% solvent yellow 33 in petrolatum	Dermatitis of scalp; sensitivity to solvent yellow 33 confirmed with patch test	Monk 1987

Effects in animals were also discussed and are summarised in the following tables, reproduced from the report. Numerous toxicity studies have been conducted in animals on solvent yellow 33 and the authors of the report considered that results indicated that solvent yellow 33 was not acutely toxic.

**TABLE C-2** Summary of Toxicity Studies Conducted with Solvent Yellow 33

Study Type	Species	Exposure Conditions	End Points and Comments	Reference
Acute toxicity	Rat, Sprague-Dawley, M, F	Oral, acute, 5 g/kg	0/10 died at 14 d, LD <sub>50</sub> > 5 g/kg	Manthei et al. 1983
Acute toxicity	Rat, Albino, M	Oral, acute, 0.3-10.0 g/kg	0/5 died at 7 d	Krebs 1980
Acute toxicity	Dog, M, F	Oral, acute, 0.3-10.0 g/kg	0/2 died at 7 d	Krebs 1980
Acute toxicity	Rabbit, New Zealand White, M or F	Dermal, acute, 2 g/kg for 24 hr	0/10 died at 14 d, LD <sub>50</sub> > 2 g/kg	Manthei et al. 1983
Repeated toxicity	Rat, Fischer 344, M, F	Inhalation, acute, 1.0 g/m <sup>3</sup> (1 hr), 1.0 g/m <sup>3</sup> (6 hr), 12.1 g/m <sup>3</sup> (6 hr) for 5 d	0/6 died at 5 d; nasal lesions	Henderson et al. 1985
Eye irritation	Rabbit, New Zealand White, M or F	0.1 g/eye	0/6 responded at 24, 48, 72 hr, 7 d; negative	Manthei et al. 1983
Dermal irritation	Rabbit, New Zealand White, M or F	Clipped skin, 0.05 g/kg for 24 hr	Primary irritation score 0.085; very mild irritant	Manthei et al. 1983
Sensitization	Guinea pig, Hartley, M	Injected 0.001 g/d for 22 days	Negative	Manthei et al. 1983
Sensitization	Guinea pig, Hartley, F	Induction: injected 5, 25, 50 µg; challenge: injected 5, 25, 50 µg	Delayed-type hypersensitivity: strong (50 µg), moderate (25 µg), weak (10 µg); cellular inflammatory response	Palazzolo and DiPasquale 1983
Subchronic toxicity	Rat	Inhalation, 0.01, 0.05, 0.2 g/m <sup>3</sup> , 6 hr/d, 5 d for 4 wk	At highest concentration, reduced body weight and respiratory effects; no histological lesions	Henderson et al. 1984
Sensitization	Guinea pig	Dermal application of 1%, 3%, 10% suspension in alcohol	Sensitizer (10% dose group)	Lamson et al. 1982

**TABLE C-2** (Continued)

Study Type	Species	Exposure Conditions	End Points and Comments	Reference
Subchronic toxicity	Rat, Albino, M	0.1-3.0% in feed, daily for 13 wk	No gross findings; growth depression at all doses; pigment accumulation in liver and kidney	Krebs 1980
Subchronic toxicity	Rat, F344/N, M, F	500-50,000 ppm in feed, daily for 13 wk	No mortality, reduced body weights in 17,000- and 50,000-ppm groups; hepatocyte degeneration at 1,700 ppm and higher, and hyaline droplets in renal cells	Eastin et al. 1996
Subchronic toxicity	Mouse, B6C3F <sub>1</sub> , M, F	500-50,000 ppm in feed, daily for 13 wk	No mortality; hepatocyte degeneration at 5,000 ppm and higher	Eastin et al. 1996
Subchronic toxicity	Rat, Fischer 344, M, F	Inhalation, 0.01, 0.05, 0.2 g/m <sup>3</sup> , 6 hr/d, 5 d/wk for 4 wk	Mild respiratory-function changes at 0.2 g/m <sup>3</sup> ; no histopathological lesions	Sun et al. 1987
Subchronic toxicity	Rat, Fischer 344, M, F	Inhalation, 0.001, 0.01, 0.1 g/m <sup>3</sup> , 6 hr/d, 5 d/wk for 13 wk	Foamy macrophages in lungs at 0.1 g/m <sup>3</sup> ; NOAEL = 0.01 g/m <sup>3</sup>	Sun et al. 1987
Subchronic toxicity	Rabbits, sex/strain not specified	0.1% and 1.0% dermal application to abdominal skin; 5 d/wk, 15 or 64 total applications (total dose not specified)	Negative for skin irritation, negative hematology and urinalysis, pigmentation in liver and kidneys	Krebs 1980
Chronic toxicity	Rat, Albino, M, F	0.03-1.0% in feed, daily for 1 yr	Suppression of growth rate; extensive pigment deposition in liver and kidneys	Krebs 1980
Chronic toxicity	Dogs, M, F	Oral, 0.05 and 0.3 g/kg/d in feed, 0.03%, for 1 yr	Moderate anemia; deposition of pigment in liver and kidney; bile-duct proliferation, moderate thyroiditis	Krebs 1980
Reproductive and developmental toxicity	Rat, F344/N, F	5,000, 17,000, or 50,000 ppm in feed, from 4 wk before breeding until 4 wk after birth	No effect on fertility, gestation length, litter size, pup birth weights	Eastin et al. 1996

Abbreviations: M, male; F, female; NOAEL, no-observed-adverse-effect level.

Studies testing solvent yellow 33 for mutagenicity were considered inconclusive. These studies are summarised in the following table.

**TABLE C-3** Summary of Mutagenicity Studies Conducted with Solvent Yellow 33

Experimental System	Exposure Conditions	End Points and Comments	Reference
Mouse, Swiss albino, male	i.p. injections, 0.001, 0.01, 0.1 g/kg, at 30 hr and 6 hr before euthanasia	No increase in micronucleated-femoral polychromatic erythrocytes	Manthei et al. 1983
Sister chromatid exchange in Chinese hamster ovary cells	0-0.04 g/mL, 3 hr	Not mutagenic or clastogenic, cytotoxic, increased sister-chromatid-exchange frequency	Brooks et al. 1989
Sister chromatid exchange in C57Bl/6J mice	0.005-0.04 g/kg, i.p., 1 injection given 25-29 hr before euthanasia	Not mutagenic, no increase in the number of sister chromatid exchanges per cell	Moore et al. 1988
<i>Salmonella typhimurium</i> (strains TA1535, TA1537, TA 1538, TA98, TA100) $\pm$ S9	0.1-1000 $\mu$ g/plate	Not mutagenic	Manthei et al. 1983
<i>Salmonella typhimurium</i> (strains TA 1535, TA1537, TA1538, TA98, TA100, TA102, TA104) $\pm$ S9	0-300 $\mu$ g/plate	Weakly positive in TA100 +S9 and TA104 $\pm$ S9; positive in TA102 $\pm$ S9	Moore et al. 1988
<i>Salmonella typhimurium</i> (strains TA1535, TA1538, TA98, TA100) $\pm$ S9	0-800 $\mu$ g/plate	Not mutagenic	Brooks et al. 1989
Mouse lymphoma cells	0-50 $\mu$ g/mL	Positive with ( $\sim$ 150-500 mutants/ $10^6$ cells) and without ( $\sim$ 400-1,000 mutants/ $10^6$ cells) S9 activation	Moore et al. 1988

Abbreviations: i.p., intraperitoneal.

CCRIS database reported the results of further mutagenicity studies for solvent yellow 33.<sup>104</sup> These were contradictory. The Ames *Salmonella Typhimurium* test system was used and several strains were tested both without and with metabolic activation. Marrs et al (1988) found that strain TA 1537 R+ was positive, without and with metabolic activation. Zeiger et al (1988) found that strain TA 1538 was positive with metabolic activation. The National Toxicology Program (1997) found strains TA 98 and TA 100 positive with metabolic activation, although Zeiger et al (1988) did not. However Zeiger et al (1988) used hamster or rat liver, S9, aroclor 1254, at 5% or 10%, while the NTP (1997) used 30%. Both Zeiger et al (1988) and the NTP (1997) found that the same strains TA 98 and TA 100 were negative, in the absence of metabolic activation. Zeiger et al (1988) also found that strains TA 97 and TA 1535 were negative with and without metabolic activation.

Marrs et al (1989) mentioned an earlier study by Moore et al (1985) that found that a mixture of solvent yellow 33 and solvent green 3 was positive in Ames strains TA102 and TA104, with and without S9 activation.<sup>105</sup>

Stoner (1985) studied solvent yellow 33 and a mixture of solvent yellow 33 and solvent green 3 for carcinogenic activity via the strain A mouse lung tumour bioassay.<sup>106</sup> At total doses of 600, 300 and 120 mg/kg, solvent yellow 33 and a mixture of solvent yellow 33 and solvent green 3 did not produce an increase in the lung tumour response (ie the percentage of mice with lung tumours or the mean number of lung tumours per mouse) when compared to controls. At autopsy, significant amounts of both dyes were observed in tissue.

<sup>104</sup> CCRIS Database. For solvent yellow 33. Accessed at <http://toxnet.nlm.nih.gov> on 25<sup>th</sup> July 2003

<sup>105</sup> Marrs T, Colgrave H, Rice P, et al (1989). The repeated dose toxicity of a smoke containing disperse blue 180, an anthraquinone dye mixture. *Journal of Hazardous Materials* 21: 73-88. ID28303

<sup>106</sup> Stoner G (1985). Bioassay of military relevant compounds for carcinogenic activity by the strain A mouse lung tumour bioassay. (Abstract). Report number AD-A156772/6. Medical College of Ohio

The National Toxicology Program (1997) conducted a 2-year carcinogenicity study in F344/N rats exposed to oral solvent yellow 33.<sup>107</sup> First generation male and female rats were given solvent yellow 33 (approximately 99% pure) in feed for up to 19 weeks and then mated, and exposure of second-generation males and females began in-utero and continued for 2 years (after weaning at 28 days of age). Groups of 60 male and female rats were given 0, 500, 1700 or 5000 ppm solvent yellow 33 in feed for 105 (males) or 106 (females) weeks after weaning. 6 to 10 rats per group were evaluated at 12 months. These exposure concentrations resulted in average daily doses of approximately 25, 85 or 250 mg solvent yellow 33/ kg body weight to males and 25, 100 or 280 mg/kg to females. Survival of males given 1700 or 5000 ppm was significantly less than that of the controls, but survival of 1700 ppm females was significantly greater than controls. Mean body weights of 1700 and 5000 ppm males and females were generally lower than those of controls throughout the study. Chemical-related clinical findings included yellow discolouration of the entire body in all exposed males and females from day 1 and head swelling and oedema in 1700 and 5000 ppm males. There was some evidence of carcinogenic activity in male rats based on increased incidences of hepatocellular adenoma, renal tubule neoplasms (adenoma or carcinoma) and squamous cell neoplasms of the oral cavity (papilloma or carcinoma). There was some evidence of carcinogenic activity in female rats based on increased incidences of hepatocellular neoplasms (adenoma or carcinoma) and less certainly uncommon squamous cell carcinoma of the oral cavity. Limitations of this study included no consistent evidence of a dose response relationship and exposures began in-utero.

Non-neoplastic changes included an increased incidence of non-neoplastic liver lesions: clear cell foci, increased basophilia and granularity in the cytoplasm of hepatocytes, bile duct, hepatocyte and Kupffer cell pigmentation in males and females and mixed cell foci in male rats.<sup>108</sup> In the kidney: increased incidence of renal tubule pigmentation and transitional epithelial hyperplasia in males and females and renal tubule hyperplasia in males. The severity of nephropathy was increased in exposed males and females.

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<sup>107</sup> National Toxicology Program (1997). Toxicology and Carcinogenesis Studies of D&C Yellow No. 11 in F344/N Rats (Feed Studies). NTP TR 463. US Department of Health and Human Services. ID 28279.

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Results of neoplastic changes are summarised in the following table:

	Male 0	Male 500	Male 1700	Male 5000	Female 0	Female 500	Female 1700	Female 5000
2-year survival	19/50	20/51	8/51	2/54	22/50	26/51	37/50	23/51
Hepatocellular ad.	1/50	2/51	1/51	7/54				
Hepatocellular ad. / ca.					0/50	2/51	5/50	5/51
Renal tubule ad.	0/50	2/51	4/51	4/54				
Renal tubule ad. / ca.	0/50	2/51	5/51	4/54				
Oral cavity sq cell pap.	1/50	1/51	2/51	4/54				
Oral cavity sq cell ca.	0/50	1/51	1/51	1/54	0/50	0/51	0/50	2/51
Oral cavity sq. Cell pap. / ca.	1/50	2/51	3/51	5/54	1/50	1/51	0/50	2/51

Ad = adenoma, Ca = carcinoma, Sq. = squamous, Pap = papilloma

Results of mutagenicity tests with solvent yellow 33 in *Salmonella typhimurium* were equivocal in one study (strain TA 100 with induced rat liver S9) and were weakly positive in another study (strains TA 98 and 100 with induced rat or hamster liver S9).<sup>109</sup> Solvent yellow 33 induced sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, with and without S9. No increase in the frequency of micronucleated normochromatic erythrocytes was observed in peripheral blood samples from male and female B6C3F1 mice administered solvent yellow 33 in feed for 13 weeks.

A reproductive toxicity study involving groups of 60 male and female rats given 0, 500, 1700 or 5000 ppm solvent yellow 33 in feed for up to 19 weeks.<sup>110</sup> All rats survived until the end of the study. The duration of gestation, the average litter size, the number of live pups on days 4 and 21 and the percentage of male pups for each exposure group were similar to those of the controls. The mean body weight of exposed litters were significantly less than those of control litters on days 14 and 21; this effect was considered to be related to solvent yellow 33.

<sup>109</sup> National Toxicology Program (1997). Toxicology and Carcinogenesis Studies of D&C Yellow No. 11 in F344/N Rats (Feed Studies). NTP TR 463. US Department of Health and Human Services. ID 28279.

<sup>110</sup> National Toxicology Program (1997). Toxicology and Carcinogenesis Studies of D&C Yellow No. 11 in F344/N Rats (Feed Studies). NTP TR 463. US Department of Health and Human Services. ID 28279.

## ***Disperse Blue 180***

Marrs et al (1989) conducted an animal study on the repeated dose toxicity of a smoke containing disperse blue 180.<sup>111</sup> The formulation consisted of disperse blue 180 (48%), potassium chlorate (26%), lactose (23%) and zinc oxide (3%). This formulation is similar to that of the blue smoke formulation in the "L" series grenade, except for the absence of kaolin colloidal, zinc stearate and gum acacia powder. Disperse blue 180 was stated to be a mixture of anthraquinone dyes, a dark blue powder, with no chemical constitution number. This formulation was ignited to produce a blue smoke and female animals were exposed to the smoke for one hour per day, five days per week, for 200 exposures (42 weeks) at three different concentrations (51.5, 156.2 and 500.4 mg per cubic metre) in a static chamber. Controls were exposed to air inside the chamber. There were four groups each of 100 mice, 50 rats and 48 guinea pigs. After the last exposure, animals were observed for 10 months. A reduction in growth relative to the controls was observed during the exposure period in all three animal types and this was partially reversible, especially in mice. There was no obvious dose-related effect on survival. In the deceased animals, dose related changes appeared to be confined to the respiratory tract in all three animals [alveolar infiltration with macrophages containing granules]. Alveologenic carcinoma in deceased mice was observed in 2/39 controls, 2/29 low dose, 5/31 medium dose and 1/33 high dose animals. The most important histological finding amongst the surviving mice, was a significantly high frequency of alveologenic carcinoma in the high dose group compared to controls and evidence of a significant trend with dose: controls 6/61, low dose 6/71, medium dose 11/68, high dose 15/67,  $p = 0.05$ . The other important histological finding amongst surviving animals was alveolar and peribronchial infiltration with macrophages containing granules, especially in rats but also in mice and guinea pigs. This was significantly more frequent in exposed animals compared to controls. Disperse blue 180 was tested in the Ames Salmonella typhimurium test. The dye disperse blue 180 was mutagenic for the strain TA 1537R+, but non-mutagenic for strains TA1535, TA1537, TA1538, TA98 and TA100. The response was not affected by the presence or absence of S9 mix. The mutagenic response was observed at dye concentrations of above 6.25 to 12.5 ug/plate. The authors concluded that it was difficult to relate the concentrations of the smoke used in the present study to humans, since the smoke is used in open air. However, over short periods, the concentration of smoke used in the high dose group of animals might be experienced by humans. The authors advised some caution with this smoke, in view of the mouse lung tumours and the mutagenicity result.

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<sup>111</sup> Marrs T, Colgrave H, Rice P, et al (1989). The repeated dose toxicity of a smoke containing disperse blue 180, an anthraquinone dye mixture. Journal of Hazardous Materials 21: 73-88. ID28303