Five previous Collegium Ramazzini Statements

First Collegium Ramazzini Statement (1984)

Report on Benzidine and its Salts

Resolution of the Collegium

In spite of the massive accumulation of scientific evidence during more than three quarters of a century and the concerted efforts of many nations of over half a century, benzidine and its derivatives continue to be produced and used in many parts of the world. Thousands, perhaps hundreds of thousands of workers have been exposed to this carcinogen. They are largely unaware at the time of exposure. They remain uninformed and unaided in dealing with persistent hazardous conditions and with suffering and death that may result.

Findings

1. Benzidine and its salts, as well as benzidine-containing products, should be regarded as strong human carcinogens. The carcinogenic effect is further confirmed by animal data and supported by studies on bacteria and mammalian cells, as well as by *in vivo* and *in vitro* data on the DNA-binding and DNA-damaging capacity of benzidine.
2. Benzidine, its salts and benzidine-containing products constitute an unnecessary and preventable hazard to exposed people and these compounds and many of their products have been strictly regulated in many countries. No evidence has been offered to indicate that the general welfare would be harmed by the elimination of these chemicals from commerce.
3. The Collegium urges these examples of regulation to be followed by all countries in order to protect workers from risk of cancer of the urinary bladder and, perhaps also, of other organs. All countries should take appropriate steps to end all further human exposure.
4. The Collegium considers the practice of shifting production of benzidine and benzidine-based dyes from developed to undeveloped countries unacceptable international behaviour that raises serious moral questions about those who engage in such practices.
5. Countries that already stringently control benzidine, its salts and benzidine-containing products should request reciprocal international action, such as through the General Agreement on Tariffs and Trade, or unilaterally prohibit the import of goods manufactured with the use of benzidine and its derivatives.

6. Substituting benzidine with other related chemicals, such as 3,3' dichlorobenzidine, which may also exhibit potent carcinogenic properties, would be unnecessary and unacceptable.

The Scientific Basis for Control

Use and international distribution

The mutagenic and carcinogenic hazards of benzidine and many of its salts have been recently reviewed and summarized (Clayson and Garner, 1976; Tola, 1980; Clayson, 1981; DHHS, 1981; Walker and Gerber, 1981; Glashan, 1982; IARC, 1982a, b; Morinaga *et al.*, 1982).
The principle industrial use of benzidine has in the past been in the production of dyes and pigments in the textile, leather, printing, plastics and furniture industries in many countries. Benzidine exposure has often occurred in conjunction with exposure to other bicyclic aromatic amines. It may still be used in some countries for the same purposes. Other occupational exposures to benzidine or its salts may be in a wide variety of laboratory work (clinical, criminal, chemical, analytical laboratories and some comparable field work).
Benzidine-based dyes are produced for export in a number of countries, including Canada, Egypt, France, Mauritania, Mexico, Philippines, Poland, Republic of Korea, Romania and India (Samuels, 1981). Other countries may also be producers and exporters of these chemicals.

Human studies

An association between occupational benzidine exposure and increased incidence of cancer of the urinary bladder has been known for over 50 years; the first study where benzidine exposure specifically was mentioned was Oppenheimer's (1927). However, as early as 1921, ILO considered benzidine as an established

human carcinogen (ILO, 1921). Benzidine was one out of several bicyclic aromatic amines responsible for the increased cancer risk of urinary bladder among aniline workers observed by Rehn (1895). The world-wide spread of urinary bladder cancer may, in part, have followed the trans-national spread of the dye-stuffs industry (Hueper, 1942, 1969; Haley, 1975). Although various factors, such as smoking and bilharzia infection, play an important rôle, chemical exposures clearly add to the result.

Apart from carcinoma of the urinary bladder, papillomas of the bladder (IARC, 1982a), and other upper and lower urinary tract tumours have been observed in many studies (Goldblatt, 1949; Uebelin and Pletscher, 1954; Douillet *et al.*, 1959; Maltoni and Ghetti, 1964). In a mixed exposure situation with beta-naphthylamine, manufacturing workers in Japan (Morinaga *et al.*, 1982) also had histologically confirmed primary cancer in liver, gall bladder, bile duct, large intestine, and lung. These observations suggest that benzidine exposures together with exposures to other carcinogenic aromatic amines could represent a multipotential carcinogenic risk. However, the multipotential risks are largely unstudied. Previously exposed populations should be studied for total cancers.

Short exposures (months and a few years) to benzidine and related compounds may be sufficient (Scott, 1952; Uebelin and Pletscher, 1954; Douillet *et al.*, 1959; Morinaga *et al.*, 1982) to induce bladder tumours, as well as fairly low exposure levels (less than 0.0005 to 17.6 mg/m^3) (Zavon *et al.*, 1973).

Animal studies

Benzidine fed to mice, or given by gavage, induces histologically benign and malignant liver tumours (IARC, 1982a) and cholangiocarcinomas (Nelson *et al.*, 1982). Benzidine hydrochloride fed to pregnant mice seemed to influence latency time of benign and malignant tumours in the offspring and to increase the frequency of hepatocellular carcinomas in mice when exposed during infancy, and in adult mice when exposed from weaning through 90 weeks (Vesselinovitch, 1983). Liver tumours did also occur in mice s.c. injected with benzidine (IARC, 1981a).

Hepatocellular carcinomas or hepatomas may also be the result of benzidine or benzidine dihydrochloride feeding in rats (IARC, 1982a). Besides, mammary tumours of different histological types may be observed. Perorally administered mice (Littlefield *et al.*, 1984a) obtained dose-related hepatic cytological alterations, hyperplasia of the bile ducts, Harderian gland adenomas, and angioma of the uterus among several lesions. One part per billion of benzidine dihydrochloride in the drinking water was estimated to produce liver tumours in less than 2.23 mice per 100,000 (Littlefield *et al.*, 1984b).

Liver tumours, mammary tumours, Zymbal gland tumours, and tumours of the colon have been observed in rats s.c. injected with benzidine or benzidine sulphate (IARC, 1982a).

In one Soviet inhalation study (quoted in IARC, 1982a), benzidine seems to have produced a few tumour-bearing animals, of which leukaemias and breast fibroadenomas occurred in more than one animal.

Benzidine- and benzidine dihydrochloride-fed hamsters also developed hepatomas or liver cell tumours, and cholangiomas or cholangiomatous tumours (IARC, 1982a).

Mutagenicity studies

Benzidine congeners or benzidine hydrochloride are mutagenic towards *Salmonella typhimurium* in presence of a metabolic activation system (Lazear and Lovie, 1977; DeFlora, 1981; Bos *et al.*, 1982; IARC, 1982a). Benzidine may also be mutagenic towards yeast and *Drosophila* and may produce sperm abnormalities in mice (IARC, 1982b). Urinary excretion of mutagenic metabolites of benzidine- or benzidine dye-exposed men and laboratory animals has been found (Tanaka *et al.*, 1981; IARC, 1982a). Chinese hamster bone marrow cells showed a statistically enhanced frequency of sister chromatid exchanges when animals were exposed to benzidine dihydrochloride (Neal and Probst, 1983).

Cell transformation

A clone of BALB/3T3 mouse embryo cells underwent a dose-related malignant transformation rate in presence of benzidine (Cortesi *et al.*, 1983).

Other DNA effects

DNA adducts are formed *in vitro* and *in vivo* after exposure to benzidine (Martin and Ekers, 1980;

Martin *et al.*, 1982; Beland *et al.*, 1983), benzidine derivatives, and benzidine-based dyes (Martin *et al.*, 1983). The adduct is a N-hydroxarylamine (Beland *et al.*, 1983), probably formed via a nitrenium ion (Martin and Eker, 1980). The oxidative metabolism may be mediated via a prostaglandin endoperoxide synthetase (Zenser *et al.*, 1980). The interference with DNA causes single strand breaks (Bermudez *et al.*, 1982) in hepatocytes of benzidine-exposed rats, and induced unscheduled DNA synthesis in liver cells *in vitro* (Bermudez *et al.*, 1982; Mirsalis *et al.*, 1982).

Metabolism of benzidine and benzidine-based dyes

Benzidine is an indirect mutagen/carcinogen activated via acetylation and hydroxylation of the amino group (Morton *et al.*, 1979, 1980). A detailed scheme of benzidine activation to the ultimate carcinogen has been proposed by Brouns *et al.* (1982).

Jenkins (1978) notes the carcinogenicity of benzidine-based dyes in part from contamination of unreacted benzidine. Benzidine contamination levels of 13-15 parts per million were found in dyes from one producer. Additional industrial exposures to free benzidine-type substrates may also occur from decomposition at temperatures easily reached in textile dying and pressing.

That the hazards of using benzidine-based dyes are not entirely associated with unreacted carcinogenic bases is well established. Littlefield *et al.* (1984b) calculate that one part per billion of benzidine dihydrochloride in the drinking water of female mice produced liver tumours in less than 2.23 mice per 100,000 population.

Genin (1977) reported that after finding benzidine in the urine of rats given dyes based on this carcinogen, he examined the urine of 22 workers exposed in dying and grinding. Benzidine was found in the urine of eight.

NIOSH (1980) found benzidine in the urine of all those who worked solely with finished dyes in a dye manufacturing plant. Urinary benzidine levels exceeded that which could be attributed to residual benzidine alone.

Regulations

Benzidine and its salts have been considered carcinogenic and are reported to be strictly regulated by Australia, Belgium, Federal Republic of Germany, Finland, Italy, Japan, the Netherlands, Poland, Romania, Sweden, Switzerland, United Kingdom, United States, USSR, and Yugoslavia (IARC, 1982a).

Benzidine-based and -containing dyes are considered human carcinogens and the governments of most industrial nations hold that worker exposure should be the lowest feasible, as in the United States (DHHS, 1981), or, as in Sweden, benzidine and its salts are not allowed to be used, including products containing 0.01% or more of these chemicals (Limit values, 1981). As a consequence, in the United States, Sweden and other countries, production of benzidine has stopped and instead benzidine-based dyes are imported or substituted.

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