Toxicology of iodine: A mini review

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SUMMARY
Iodine is necessary for all living organisms. Deficiency of iodine in the organism leads to various diseases (including mental) and increased rates of cancer. It is well known that one third of the world’s population lived in iodine-deficient areas. At present time, the primary intervention for preventing iodine deficiency disorders worldwide is through the iodization of salt. The two most common types of fortificant used to iodize salt are potassium iodide and potassium iodate. Iodine-containing compounds are also widely used in clinical medicine as a highly effective topical antimicrobial agent that has been used clinically in the treatment of wounds. Hence, the genetic toxicology of iodine and iodine-containing compounds is very essential topic. In this literature review are analyzed the data concerning genetic toxicology and the influence of these compounds on tumor rates in epidemiological and experimental studies.

Key words: Iodine; Iodine Compounds; Toxicogenetics; Carcinogenicity Tests; Neoplasms

INTRODUCTION
Iodine is the required element in the diet richest in electrons. Inorganic iodides are necessary for all living vegetable and animal cells, but only the Vertebrates have the thyroid gland and its iodinated hormones. In humans, the total amount of iodine is about 15-50 mg and roughly 80 % is located in the thyroid gland (1, 2). The US Food and Nutrition Board of the Institute of Medicine has estimated a daily iodine requirement for adults of 150 µg/day and a tolerable upper intake level of 1100 µg/day (2). The upper intake level is defined as the highest level of daily iodine intake that is likely to pose no risk of adverse health effects in almost all individuals. However, intake levels considerably above the upper intake level are well tolerated by most individuals (2).

Although iodine is one of essential microelements, one third of the world’s population lived in iodine-deficient areas (3). Iodine deficiency induces:
1) goiter, including toxic nodular goiter, increased occurrence of hypothyroidism in moderate-to-severe iodine deficiency; decreased occurrence of hypothyroidism in mild iodine deficiency, increased susceptibility of the thyroid gland to nuclear radiation at all ages; 2) abortion, stillbirth, congenital anomalies, perinatal mortality in fetus; 3) infant mortality, endemic cretinism, child and adolescent; 4) impaired mental function, iodine-induced hyperthyroidism in adults (3). At present time the primary intervention for preventing iodine deficiency disorders worldwide is through the iodization of salt (4). The two most common types of fortificant used to iodize salt are potassium iodide (KI) and potassium iodate (KIO₃). In general, potassium iodate (KIO₃) is preferred because of its stability (4).

Since one-third of the world’s population lived in iodine-deficient areas (3), and they use iodine compounds supplementation in food, it is warranted to assess possible toxic effects of these compounds. The last review paper concerning toxicology of these compounds was published in 2001 (5). The aim of this mini-review was to assess possible toxic effects of iodine and iodine-containing compounds with special emphasis on genotoxicity and carcinogenicity studies.

ROLE OF IODINE AS AN ANTIOXIDANT IN EVOLUTION
Venturi et al. (6-9) proposed that over three billion years ago, Algae, which contain the highest amount of iodine, were the first living cells to produce oxygen, which was toxic at that time, in the terrestrial atmosphere. Therefore, algal cells required a protective antioxidant action in which iodides might have had this specific role. In fact iodides are greatly present and available in seawaters, where algal phytoplankton acts as a biological accumulator of iodides. Iodide is proposed to have an ancestral antioxidant function in iodide-concentrating cells in all living organisms (from primitive seaweeds to mammals) (6-9). In these cells iodide acts as an electron donor in the presence of H₂O₂ and peroxide (10), the remaining iodine readily iodinates the tyrosine and (more slowly) the histidine or some specific lipid (11), and so, neutralizes its own high oxidant power. Recently this hypothesis of the ancestral antioxidant action of iodides has experimentally been confirmed in some algae by an important study carried out by Kupper et al. (12). Researchers reported the cooperation between very strong antioxidant selenium and iodine. In fact selenium is present in peroxidase enzymes and in type 1 and type 3 deiodinases, which are able to oxidate iodides and the latter enzymes produce iodides from iodothyronines (1). Thyroid gland is the richest tissue in selenium and iodine, whose deficiencies constitute an important risk factor for thyroid morbidity and carcinogenesis (1, 6-9). Dietary iodides are able to defend brain and liver cells from lipid peroxidation in rats (13). The antioxidant action of iodides has also been described in isolated rabbit eyes (14). Rieger et al. (15), Winkler et al. (16) and Buchberger et al. (17) reported about beneficial and antioxidant actions of iodides in many chronic diseases and in eye cataractogenesis. In the Mammalia several extrathyroidal organs share the same gene expression of sodium/iodide symporter of thyroidal iodide-pump and particularly stomach mucosa and lactating mammary gland (18). Based on this study, Venturi et al. (6-9) proposed that iodine excess or deficiency could play a role in carcinogenesis of mentioned organs.

Iodine is known to have many beneficial effects on human population, such as treatment of hypothyroidism, weight loss, rheumatism, ulcers, hair loss, and maintenance of arteries, nervous tissue, and nails. To prevent iodine deficiency disorders in human population, iodine is added in the diet in the form of iodide or iodate. Table salt is generally used as a carrier to supply populations with the necessary iodine (19). In a majority of European countries, supply of iodine in the form of iodate is allowed by


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health authorities, the stability of iodate being better than the one of iodide. However in 1999, the French Academy of Medicine (20) has expressed concern about the safety of iodate for human health since this compound had not been adequately tested for genotoxicity and carcinogenicity. More recently an advice of the French Agency for Food Safety (5, 20) did not recommend the use of iodate instead of iodide for table salt supplementation, for the same reasons. In a recent review on the toxicology of iodate, (5) concluded that this compound has been used for decades as an additive to salt and bread without notable toxic effects at levels more than 100 times higher than the expected human exposure. Moreover, this compound, added to salt in low amounts, is probably reduced to iodide in food or in the body (5). In these conditions, the authors suggested that there was no urgent need to change existing program from iodate to iodide.

**ACUTE TOXICITY**

The acute toxicity of iodine (pure) in mammals is quite low. For example, in rats the oral LD₅₀ is 14,000 mg/kg, and in mice it is even lower – 22,000 mg/kg. In humans the LDLo is 28 mg/kg (www.toxnet.nlm.nih.gov). Sodium salt of iodine (NaI) is much toxic than iodine and its LD₅₀ in rats (oral treatment) is 4,340 mg/kg, and 1,000 mg/kg in mice (www.toxnet.nlm.nih.gov). Baker (2) stated that all animal species appear to have a wide margin of safety for excess iodine consumption. Dietary iodine levels of 500 to 1000 times higher than the minimal dietary required level are generally well tolerated in rats, pigs, chickens, and ruminant animals. Among the species studied, horses seem to be most susceptible to iodine toxicity (2). Studies with rabbits, hamsters, rats, pigs, and chickens suggest that rats, hamsters, pigs, and chickens can tolerate dietary iodine levels up to 500 mg/kg, but rabbits experience serious mortality in offspring when 250 mg/kg is fed to the dams in late gestation. When given intravenously to rats, or when added to whole blood or tissue homogenates in vitro or to foodstuff, iodate is quantitatively reduced to iodide by nonenzymatic reactions and thus it becomes available to the body as iodide. Therefore, except perhaps for the gastrointestinal mucosa, exposure of tissues to iodate might be minimal. At much higher doses given intravenously (i.e., above 10 mg/kg), iodate is highly toxic to the retina. Ocular toxicity in humans has occurred only after exposure to doses of 600 to 1,200 mg per individual. Oral exposures of several animal species to high doses, exceeding the human intake from fortified salt by orders of magnitude, pointed to corrosive effects in the gastrointestinal tract, hemolysis, nephrotoxicity, and hepatic injury. The studies do not meet current standards of toxicity testing, mostly because they lacked toxicokinetic data and did not separate iodate-specific effects from the effects of an overdose of any form of iodine. With regard to tissue injury, however, the data indicate a negligible risk of the small oral long-term doses achieved with iodate-fortified salt (5).

**GENOTOXICITY OF IODINE AND THE COMPOUNDS CONTAINING IODINE**

It is noteworthy that iodine itself is very reactive agent, and mostly in the nature it is present as salts - iodate and iodide (21). Genotoxic potency of I₂ was studied using the L5178Y mouse (TK+/-) lymphoma assay (21). The established mutagens ethyl methanesulfonate and dimethylnitrosamine were highly active in this assay and iodine was inactive. Furthermore, in the presence of a rat liver microsomal activation fraction (S-9), I₂ had only marginal activity as mutagens. Using the Balb/c 3T3 transformation assay, the authors assessed the transformational capacities of iodine and the positive mutagen N-methyl-N-nitro-N-nitrosoguanidine (MNNG). All concentrations of iodine were inactive in this assay. It was concluded from these studies that I₂ did not possess any biologically significant mutagenic or cell transforming ability (21). Bürgi et al. (5) concluded that additional genotoxicity data are needed for complete risk assessment of the iodine containing compounds. Indeed, the limited existing data on the genotoxicity of iodate seem to be insufficient. It was reported (22, 23) that iodate caused no mutagenic action in *Salmonella typhimurium* TA98 and TA100 strains with and without metabolic activation. Iodide was found negative in the mouse bone marrow micronucleus (MN) test even at high, close to toxic doses and in the recessive lethal mutation assay in Drosophila melanogaster. Comparative studies of the oxidative properties of iodate and other halogenate salts (bromate and chloride) have indirectly suggested that iodate would be of low, if any genotoxic potential (22, 24, 25). By contrast, bromate, a halogenate salt analogue, induced significant increase in the frequency of MNed cells in murine bone marrow, and chlorate has been proved to be mutagenic in bacteria *Salmonella typhimurium* TA98 and TA100 strains with and without the metabolic activation in the recessive lethal mutation assay in Drosophila (23, 25). Potassium iodate was studied in CHO cells by means of MN assay and the comet assay (26), and potassium iodide in the L5178Y mouse (TK+/-) lymphoma assay and the Balb/c 3T3 transformation assay (21). The results of these studies showed all applied assays failed to detect any evidence of genotoxicity of both compounds at concentrations up to 10 mM (close to cytotoxic concentrations). These data confirm the results obtained by other investigators (23, 25). At the same time the authors confirmed high genotoxic potency of potassium bromate to damage DNA. In DNA-repair test it was 2-fold higher than that of positive control (etoposide) at 0 h, 4-fold higher at 0.5 h and 1 h, and 6-fold at 24 h. Potassium chloride was not active in these conditions, like iodide. It should be noted that potassium bromate was found positive in *Salmonella typhimurium* and chromosome aberrations (CA) and MN assays in rats and mice (27). Some other halogens salts (such as chlorine dioxide, sodium chlorite) were found active in MN assay in mice (28). Based only on the data on genotoxicity of few halogen salts, some investigators hesitate if iodine, as halogen, could have carcinogenic and genotoxic properties. Earlier Parsons and Chipman (22) showed that bromate, but not chlorate and iodate can induce oxidized DNA bases. They found that bromate could induce lipid peroxidation processes in cells with subsequent secondary alterations of DNA. Recently Hikiba et al. (29) studied iodofuran and iodine induced CA in Syrian hamster embryo cells (SHEC). Iodofuran even at high concentrations (up to 800 μM) did not induce cytogenetic disturbances both in the experiments with and without metabolic activation. Pure iodine dissolved in DMSO, induced CA in SHEC only at concentrations of 600 μM and 800 μM, 13.0% and 19.0% of aberrant metaphases, respectively. Iodine was not active at concentration 400 μM. These chemical agents were studied in frames of investigations of agents used in dental practice. Some other agents used in dental practice,
such as carbol camphor, formaldehyde, guaiacol, and zinc oxide showed much higher cytogenetic activity (63% - 71% of aberrant cells).

Iodine at high concentrations, close to toxic ones (600 μM and 800 μM), induced SCEs (sister chromatide exchanges) (30), but not unscheduled DNA synthesis in SHED (31). Miyachi and Tsutsui repeated experiment in SHED on the ability of iodine to induce SCE and found slight but significant difference from the control activity (32). Iodine was active only at high doses close to toxic ones (600 μM and 800 μM). Based on the data of activity of iodine in CA and SCE assays in SHED, Miyachi and Tsutsui (32) proposed that the application of iodine in dental practice could be a source of cytogenetic danger.

Povidone-iodine genotoxicity was studied in human blood in vitro by means of SCE (33). It induced substantial decrease in antioxidant enzymes level, but failed to induce genetic damage (increase of rates of SCE) (33). It induced substantial decrease in antioxidant enzymes level, but failed to induce genetic damage.

The comet assay and the CA test were used to characterize the genotypic activity of povidone-iodine within 4 h of contact with the cells. Methyl methanesulphonate without metabolic activation and benz[a]pyrene activated with S9 mix were used as positive controls. All tested concentrations of povidone-iodine formulations (4–6% Repithel®, 3–5% Betasidonona® ointment and 1–2% Betasidonona® solution) did not damage DNA in the comet assay. At concentrations of 10% Repithel®, 5% Betasidonona® ointment and 2.5% Betasidonona® solution in culture medium (60% cytotoxicity within 4 h of contact with CHO-K1 cells), no significant increase in the CA levels compared with negative control was observed. Hence, all tested concentrations of povidone-iodine formulations were not genotoxic in the CA test and the comet assay (34).

The cytotoxicity of iodine-containing drugs was relatively low – after 30 min contact time using vital dye neutral red, the following IC50 were determined: 16–18% Repithel®, 8–9% Betasidonona® ointment and 1.8–2% Betasidonona® solution (35). The authors conclude that the first attack of iodine to mammalian cells is carried out on enzymes, perhaps by oxidation, followed by membrane attack (35). These results confirmed the data obtained earlier by Niedler (36) that povidone-iodine possess low cytotoxicity in mammalian cells.

Iodide and Cu salts of iodine were not active in chromosome assay in cytotoxicity in mammalian cells. The established mutagens ethyl methanesulfonate and dimethylnitrosamine were highly active in this assay, whereas iodine-containing compounds were inactive. Furthermore, in the presence of a rat liver microsomal activating fraction (S-9), povidone-iodine had only marginal activity as mutagens. Using the Balb/c 3T3 transformation assay the authors assessed the transformational capacities of these same agents and the positive mutagen N-methyl-N-nitro-N-nitrosoguanidine (MNNG). All concentrations of the iodine compounds tested were inactive in this assay. It was concluded from these studies that polyvinyl pyrrolidine and povidone-iodine did not possess any biologically significant mutagenic or cell transforming ability (21).

CARCINOGENICITY OF IODINE AND THE COMPOUNDS CONTAINING IODINE

Iodine is an essential nutrient for the normal growth and development of humans and animals and is necessary for normal metabolism and regulation of thyroid hormones. Male F344/Ncr rats fed iodine deficient diet received a single IV injection of N-nitrosomethylurea. The results of this experiment provided evidence that the iodine-deficient diet is a potent promoter of thyroid tumors initiated by N-nitrosomethylurea and carcinogenic by itself (45). These data were confirmed by Kanno et al. (46) who showed that iodine excess produced colloid goiter, characterized by large follicles with flat epithelium and abundant colloid admixed with normal or...
small-sized follicles lined by epithelium of normal height, together with normal serum T4 and slightly decreased TSH. There is only one study in the literature concerning ability of iodine to transform cultured mammalian cells (Syrian golden hamster cells) at high, close to toxic concentrations (30). Both iodine deficiency and excess are promoters of male F344 rats thyroid neoplasia induced by a single subcutaneous injection of 2,800 mg/kg of N-bis(2-hydroxypropyl)-nitrosamine (46). The same regularity was found also in epidemiological studies. Because of that the World Cancer Research Found and the American Institute for Cancer Research reported that dietary iodine deficiency and also excess are tumor promoters and carcinogens in the thyroid gland (47).

Exclusively during pregnancy and lactation, which are considered protective conditions against breast cancer, the mammary gland has a high, but temporary ability in concentrating iodides and also in forming iodo-proteins in alveolar and ductular cells by specific peroxidase (48). When female rats are kept iodine-deficient, atrophy and necrosis takes place in the mammary gland and areas of dysplasia and atypia are seen, and in pregnant mice, mammary tumor cells lose their ability to iodinate (1). Eskin (49, 50) and Edington (51) reported that iodine deficiency causes breast dysplasia and cancer in rats and probably in humans, and showed a mammary tumor reduction in rats after iodine treatment. There are also some epidemiological evidences concerning the role of iodine metabolism disorders and breast cancer (52). The coincidence of thyroid disorders and breast cancer has long been a subject of debate. Associations with hyperthyroidism, hypothyroidism, thyroiditis, and nontoxic goiter have been reported. However, no convincing evidence exists of a causal role for overt thyroid disease in breast cancer. Geographical variations in the incidence of breast cancer have been attributed to differences in dietary iodine intake and an effect of iodide on the breast has been postulated. Recent reports have shown a direct association between thyroid enlargement, as assessed by ultrasound, and breast cancer. Although the exact mechanism for the demonstrated association between diseases of the thyroid and breast cancer remains to be elucidated, there is at least the possibility that the presence of thyroid abnormalities may influence breast cancer progression and this alone should stimulate awareness into the coincidence of the two disorders (53, 54). Analysis of the data concerning breast cancer and iodine in humans and rodents let Aceves et al. (55) call iodine as gatekeeper of mammary gland.

Analyzing the associations between the incidence of gastric cancer and iodine consumption in Italy, Venturi et al. (1, 6) showed that there is a correlation – gastric cancer, thyroid cancer and goiter are statistically correlated and more frequent in iodine deficient areas, such as in Alpine and Apeninnes valleys, and in regions of northern and central Italy compared to southern Italy where the majority of the population lives in sea-side areas. It is noteworthy that both iodine excess and deficiency might constitute a risk factor for gastric cancer and atrophic gastritis. The data of epidemiological studies of coastal population of China and Japan with the highest in the world consumption of iodine (200 mg/daily/ per person due to excessive and harmful consumption of marine algae – sea-weeds) showed the highest rates of gastric cancer incidence (1). The data of Venturi et al. (1, 6) concerning the relation of iodine and gastric cancer were confirmed in study of Turkish investigators (56). A case-control study by Key et al. (57) found an inverse trend between iodine intake and prostate cancer risk. Hoption Cann et al. (58) stated that further studies are needed for firm conclusions concerning prostate cancer and dietary iodine.

Hoption Cann et al. (58) further examined the relationship between thyroid disease and prostate cancer risk. Relative to the group with low urinary iodine, the age-adjusted hazard ratio was higher. Since dietary iodine plays an important role in thyroid diseases, it could also play a role in prostate carcinogenesis.

Based on the epidemiological data, Stadel (59) stated that geographic differences in the rates of breast, endometrial, and ovarian cancer appear to be inversely correlated with dietary iodine intake. Endocrinological considerations suggest that a low dietary iodine intake may produce a state of increased effective gonadotrophin stimulation, which in turn may produce a hyperestrogenic state characterized by relatively high production of estrone and estradiol and a relatively low estradiol to estrone plus estradiol ratio. This altered endocrine state may increase the risk of breast, endometrial, and ovarian cancer. Increasing dietary iodine intake may reduce the risk of these cancers (59).

**CARCINOGENICITY OF IODINE AND THE COMPOUNDS CONTAINING IODINE**

Some compounds containing iodine were tested for carcinogenicity (38, 39, 60). Takegawa et al. (60) studied possible carcinogenic activity of potassium iodide given in drinking water in male and female F344/DuCrj rats. The compound was given at concentrations of 10, 100 and 1000 ppm for 104 weeks (total intake was 402, 3668 and 38609 mg/kg/104 weeks, respectively). Only in male rats higher doses of the compounds decreased the survival rates. No significant difference was found in treated female rats compared with the control. Squamous cells carcinoma was found in 4 out of 40 male rats and in 3 out of 40 female rats. No compound related induction of any lesion was apparent in other organs or tissues. The authors suggested that potassium iodide has weak carcinogenic potency and induces tumors via epigenetic mechanisms only at very high doses (60).

Methyl iodide is formed principally in the oceans. Smaller amounts are produced industrially for use as a chemical intermediate. Exposures occur from occupational use and from ubiquitous low-level exposure in ambient air and in water. Methyl iodide was tested for carcinogenicity in one experiment in rats by subcutaneous administration and in a screening test for lung adenomas in strain A mice by intraperitoneal injection. It induced local sarcomas in rats after single or repeated subcutaneous injections; a marginally increased incidence of lung tumors was observed in mice. No data were available to evaluate the reproductive effects or prenatal toxicity of methyl iodide to experimental animals (38, 39). Methyl iodide induces DNA damage and is mutagenic to bacteria in the presence or absence of an exogenous metabolic system. It induces mitotic recombination in yeast and mutations in cultured mammalian cells. It induces transformation in Syrian hamster embryo cells but not in C3H 101T1/2 cells (38, 39). The experts of IARC classified it as group 3 agent (not classified for human carcinogenicity). Toxicology and carcinogenesis studies of iiodinated
seaweed extracts induced apoptosis in 3 kind of human breast cancer cell lines, but not human normal breast cells. But some investigations hesitated if these effects are exclusively due to iodine and not to other physiologically active components containing in seaweeds (66). Garcia-Solis et al. (67) analyzed the effect of molecular iodine (I\textsubscript{2}) on the induction and promotion of mammary cancer induced by N-methyl-N-nitrosourea in virgin Sprague-Dawley rats. Continuous I\textsubscript{2} treated rats exhibited a strong and persistent reduction in mammary cancer incidence (30%) compared to controls (72.7%). The protective effect of I\textsubscript{2} was correlated with the lowest levels of lipoperoxidation expression in mammary glands. p53 expression did not show any changes. A potent antineoplastic effect of I\textsubscript{2} on the progression of mammary cancer may be related to a decrease in the oxidative cell environment (67).

Soriano et al. (68) analyzed the effect of various concentrations of iodine and/or iodide in the dimethylbenz[a]anthracene (DMBA) mammary cancer model in rats. The results show that 0.1% iodine or iodide increases the expression of peroxisome proliferator-activated receptor type γ (PPAR\textsubscript{γ}) by means of triggering caspase-mediated apoptosis pathways in malignant mammary gland tissue, but not in normal mammary gland tissues. DMBA treatment induces the expression of lactoperoxidase, which participates in the antineoplastic effect of iodide and could be involved in the proneoplastic effect of estrogens, increasing the formation of DNA adducts. The results of the study show that a supplement of 0.1% molecular iodine/potassium iodide (0.05/0.05%) induces antitumor effects, preventing estrogen-induced DNA adducts and impels apoptosis through PPAR\textsubscript{γ}/caspases in both precanancerous and tumor cells. Since this iodine concentration does not modify the cytology (histology, apoptosis rate) or physiology (triiodothyronine and thyrotropin) of the thyroid gland, the authors propose that it be considered as an adjuvant treatment for premenopausal mammary cancer (68).

**CONCLUSION**

Based on the absence of genotoxicity data of iodine and iodine-containing compounds in most short-term tests (the Ames assay, Drosophila-mutagenicity study, MN assay in bone marrow cells of mice and CHO cells, the comet assay) (21, 22, 24, 25), it can be concluded that they hardly have carcinogenic potency. Positive results were obtained only in Syrian embryo hamster cells in SCE and CA tests but only when iodine was applied at very high concentrations close to the toxic ones (600 and 800 mM) (32). The only one iodine–containing compound, KI, was studied in long-term carcinogenicity study in rats (60). Only at highest applied dose (38.6 mg/kg/104 weeks) and only in male rats tumors of salivary glands were observed in 10% animals (60). In contrast, iodine decreases significantly (from 73% to 30%) tumor rates induced by mammary carcinogen, N-methyl-N-nitrosourea, in female rats. Based on aforementioned data, it can be concluded that:

1. Iodine and iodine-containing compounds are not genotoxic in the most short-term tests, and it is indirect evidence of absence of carcinogenic activity of these compounds with high probability.

2. Iodine possibly supports antcarcinogenic defense of the organism at physiological doses and in some circumstances acts as an antcarcinogenic agent.
3. Some data evidenced that iodine can prevent breast and gastric cancer in humans (iodine even has been called as gatekeeper of mammary gland integrity).

4. Supplementation of food with iodine can be considered as safe from point of view of genetic toxicology.

Conflicts of Interest
We declare no conflicts of interest.

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