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Review

The carcinogenic effects of benzoquinones produced by the flour beetle

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Abstract

Humans and animals come into contact with various compounds in their natural environment. Most of the encountered substances are neutral, yet some may carry adverse health effects. The ingested food may be a source of harmful substances, including benzoquinones which, as shown by research results cited in this paper, demonstrate toxic, carcinogenic and enterotoxic activity. This group of compounds is inclusive of 2-methyl-1,4-benzoquinone (MBQ) and 2-ethyl-1,4-benzoquinone (EBQ), defensive secretions of the confused flour beetle (*Tribolium confusum* J. du V) and the red flour beetle (*Tribolium castaneum* Herbst). Benzoquinones have a carcinogenic effect, they are inhibitors of growth of various microorganisms, they produce a self-defense mechanism in threat situations and affect population aggregation. As noted by the referenced authors, the properties of benzoquinones have not been fully researched to this date.

Key words: benzoquinones, flour beetles, quinone carcinogenicity

Introduction

Flour beetles, including the confused flour beetle (*Tribolium confusum*) and the red flour beetle (*Tribolium castaneum*), belong to the family of darkling beetles (*Tenebrionidae*) of the order *Coleoptera*. Flour beetles are small insects with body length of 3-5 mm. The head, pronotum and elytrae are shiny, red-brown to dark brown in color. Coloring changes with age. Minor differences are observed between species. The antennae are composed of 11 segments. In the confused flour beetle, the antennae become wider from the fifth segment, whereas in the red flour beetle the last segments form a club. Eye setting is also a discriminating feature. When viewed from the ventral side, the eyes of the confused flour beetle are set wider than the eyes of red flour beetle. Both species have well-formed flight wings, but only the red flour beetle flies in warm interiors or in a tropical climate. The flour beetle develops at a temperature of 28-38°C (optimal 30-33°C) and humidity of 10-100% (optimal 70%). The length of developmental cycle depends on environmental conditions. At 24°C it lasts 54 days, and at 34°C – 26 days. Flour beetles may survive for more than two weeks without nutrition. They live 2-3 or even 4 years. The female lays around 15 eggs per day, a total of 1200 eggs in her lifetime. White-colored eggs with food particles adhering to the surface have the length of 0.6-0.7 mm and the width of 0.3-0.4 mm, and they hatch into larvae. The larvae go through 5-18 growth stages (7-8 on average). Adult larvae emerge on the surface of infested prod-

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ucts where they undergo metamorphosis. The pupae do not feed at this stage, and adults emerge within a few days (Roth and Howland 1941).

Flour beetles are among the most common pest insects found in stored grain and milled products. Both adults and larvae feed on kernels, causing damage to the seed and rendering it unfit for sowing purposes. Flour beetles produce large quantities of dust which prevents seed respiration, contributes to seed overheating, excessive moisture, the growth of mycotoxic fungi, bacteria and acarid mites.

Beetles have defensive glands which secret quinones such as 2-methyl-p-benzoquinone, 2-ethyl-p-benzoquionone, hydroquinone (Alexander and Barton 1943, Loconti and Roth 1953, Engelhardt et al. 1965, Ladisch et al. 1967, Blum 1978, Howard 1987), commonly referred to as benzoquinones. The darkling beetle family is the most prolific source of 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone (Omaye et al. 1981). An individual beetle secrets from several micrograms to 0.5 mg of quinones into the substrate (grain, flour, cereal) (Ladisch and Mcque 1953, Yezerski et al. 2000, 2004). The insects rapidly colonize the infested food, and quinone concentrations are very high in populations of thousands of individuals.

Benzoquinones give grain a characteristic, unpleasant odor, and infested flour has a pinkish color (Payne 1925). In 1941, Roth and Howland isolated the odor excretion from *T. confusum* by passing dry air over the beetles. The excretion was condensed in a dry ice trap, yielding yellow-brown crystals. At room temperature, the collected substance was a volatile liquid with a pungent quinone odor that easily reacted with KI, H_2SO_4 and the starch solution, turning blue-black in color.

As noted by Roth (1943), odor secreting glands on the abdomen and thorax of adult *Tribolium castaneum*, *T. confusum* and *T. destructor* (Uyttenb.) beetles contain a pungent, irritating fluid. In a paper published in 1943, Alexander and Barton reported on ethylquinone secretions in the flour beetle. In 1971, Von Endt and Wheeler isolated a new compound, 1-pentadecene, in addition to the previously determined 2-methyl-1,4-benzoquinone and 2-ethyl-1,4--benzoquinone produced by *Tribolium confusum*, volatile compounds that are stored in special containers. The aliphatic ketone $C_{14}H_{26}O$ had not been previously investigated. 1-pentadecene facilitates absorption of the admixed quinones by *Tribolium* enemies. Howard (1987) performed chemosystematic analysis of defensive secretions in 10 insect groups from the darkling beetle family (*Tenebrionidae*), including of the genus *Tribolium* represented by the confused flour beetle and the red flour beetle. The author determined the presence of 15 hydrocarbons, of which five were found in all studied insects, including 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone.

The specific composition of 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone and the methods of extracting these compounds from *T. castaneum* and *T. confusum* were determined by Pappas and Morrison (1995). The proposed methods were further modified by Pappas and Wardrop (1996). Insects were kept in methanol (HPLC purity) and analyzed by UV spectroscopy, reversed-phase HPLC and GC/MS. Methanol solutions obtained from both beetle species contained methyl-1,4-benzoquinone (MBQ) and ethyl-1,4-benzoquinone (EBQ). No significant differences in MBQ quantities were determined between samples. Significantly higher quantities of EBQ and the MBQ and EBQ mixture were found in *T. castaneum* than in *T. confusum*. The above authors concluded that the applied methods for the isolation, identification and quantitative determination of benzoquinone were relatively simple, quick and accurate for determining benzoquinone secretions from individual beetles.

Structure

Benzoquinones are chemical derivatives of benzenes. They are cyclic organic unsaturated diketone compounds ($C_6H_4O_2$) that contain two carbonyl groups (C=O). Benzoquinones are readily soluble in organic solvents. Benzoquinones have bright yellow, orange or red color and a pungent odor. They are commonly found in plants and animals as biologically active substances, and they are classified into three groups: monocyclic, including 1,4-benzoquinone and benzoquinones secreted by the flour beetle, bicyclic naphthoquinones, including vitamin K, and tricyclic anthraquinones, such as alizarin dye.

1,4-benzoquinone has CAS registry number 106-51-4, systematic name: 2,5-cyclohexadiene--1,4-dione, synonym: para-benzoquinone. It is a solid substance with bright yellow color, pungent odor, and it sublimes even at room temperature. The International Agency for Research on Cancer (IARC 1999) has classified 1,4-benzoquinone as a Group 3 substance:

– Which is not classifiable as to its carcinogenicity to humans and for which the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals;

– For which the evidence of carcinogenicity is inadequate in humans but sufficient in experimental animals, and substances may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Absorption and metabolism

Benzoquinone is readily absorbed from the gastrointestinal tract and subcutaneous tissue. It is excreted partially unchanged and partly as hydroquinone, the major proportion of which is eliminated as acid conjugates. The application of benzoquinone causes local skin changes, including discoloration, erythema and the appearance of papules; necrosis can occur. Exposure to vapors induces serious vision disturbances; injury extends through the entire conjunctiva and cornea (IARC 1977).

1,4-benzoquinone depresses cellular respiration in tissues under *in vitro* conditions. Large doses induce local paralysis, clonic convulsions, decreased blood pressure and death due to paralysis of the medullary centers. Signs of kidney damage were observed in severely poisoned animals (IARC 1977). Benzoquinone is a metabolite of benzene which, if ingested, undergoes a series of transformations and reactions that produce more toxic compounds than the original substance (Monks and Jones 2002).

Benzene's mechanism of toxicity has been studied extensively. Post et al. (1985) investigated benzene metabolism and the effect of benzene, p-benzoquinone and hydroquinone metabolites on the body structure. Their findings suggest that benzene may adversely affect the bone marrow stroma, resulting in defective hematopoietic support. The above authors pointed to a correlation between benzene's toxicity and its effect on macrophages in the bone marrow stroma, the main source of protein factors supporting the proliferation and differentiation of precursor cells. They investigated benzene's effect on the synthesis of macrophage RNA. Unlike phenol, the main benzene metabolite in the bone marrow, benzene itself is not metabolized in macrophages. Benzene is transformed by peroxidation into free metabolites and their derivatives which covalently bind to cellular macromolecules (proteins).

Post et al. (1984) used benzene, hydroquinone and p-benzoquinone metabolites to inhibit mRNA synthesis in the nuclei of bone marrow cells in rabbits. The inhibition process is correlated with IC_{50} values for both 6×10^{6}M compounds. Phenol did not inhibit mRNA synthesis at a concentration of 10^{-3}M, and the above was attributed to the fact that only benzene metabolites have an inhibitory effect on bone marrow cells. An *in vitro* study of mouse spleen lymphocytes demonstrated the inhibitory effect of p-benzoquinone (PBQ), a benzene metabolite. Already at low concentrations of 5 μM, PBQ inhibited lymphocyte-stimulating factor IL-2 without affecting lymphocyte viability. The above suggests that benzene exposure can cause aplastic anemia (Post et al. 1985).

Benzene and its metabolites inhibit RNA synthesis subject to the applied dose, at 50% inhibitory concentration of 5×10^{-3}M for benzene, 2.5×10^{-3}M for phenol, 2.5×10^{-5}M for hydroquinone and 6×10^{-6}M for p-benzoquinone; the phenomenon observed did not result from impaired cell viability. RNA synthesis in macrophages is most probably inhibited by benzene which probably blocks uridine transport in macrophages, and phenol which inhibits their conversion into covalent bond forms. The above suggests that benzene and phenol may inhibit the synthesis of hematopoietic colony-stimulating factors (Post et al. 1986).

D'Odorico et al. (1997) demonstrated that synthetic quinones such as menadione cause DNA damage, most probably with the involvement of free radicals in the redox cycle. They suggested that synthetic quinones may induce neoplasm formation in various locations. Using human colon adenocarcinoma grade II cell line HT-29, they investigated the extent of DNA damage induced by menadione and vitamins K1 and K2. Menadione caused significant DNA damage at low concentrations (25-200 μM) in linear correlation $r = 0.95$. In the presence of dicoumarol, the inhibitor of DT-diaphorase, signs of damage were observed at concentrations five-fold lower, indicating that free radicals produced in the redox cycle play a key role. The reported results suggest that despite their participation in the redox cycle, natural forms of vitamin K do not cause damage to DNA in HT-29 cells.

Souček et al. (2000) investigated mutual transformations of hydroquinone, semiquinone and benzoquinone in relation to spontaneous and enzymatic processes. Their findings suggest that hydroquinone and benzoquinone stimulate the formation of OH free radicals in microsomes and inhibit lipid peroxidation. Other authors (Souček et al. 1999, Kondrová et al. 2007, Zhongwen Xie et al. 2005) studied reactive benzene metabolites – 1,4-benzoquinone and 1,4-hydroquinone, and the effects of their toxic activity resulting from covalent and/or oxidative damage, binding to DNA and proteins. Efforts were also made to investigate the mechanisms by which catechol, 1,4-hydroquinine and 1,4-benzoquinine damage cytochrome P450 and induce oxidative stress in liver microsomes in guinea pigs. In the group of the studied metabolites, 1,4-benzoquinone caused the greatest damage to cytochrome P450. Yezerski et al. (2005, 2007) reported that are inhibitors of growth of various microorganisms.

Carcinogenicity and toxicity

Takizawa (1940, 1941), Takizawa and Sugischita (1948) and Sugischita (1950) conducted pioneer research into benzoquinones in the early 1940s. Over a period of 200 days, Takizawa placed drops of p-benzoquinone dissolved in benzene at a concentration of 0.1%, 0.25% and 0% (control – benzene alone) on the skin of experimental mice every 1-2 days. In the control group of 46 animals, one mouse developed papilloma, while pulmonary adenocarcinoma was observed in two individuals. In the group of mice treated with 0.1% solution, six were diagnosed with papilloma, two animals developed skin cancer and 10 – pulmonary adenocarcinoma. In the 0.25% group (44 mice), papilloma was observed in three animals, skin cancer was found in one mouse, and pulmonary adenocarcinoma – in five individuals. Ten years later, Ladisch and Mcque (1953) developed methods for obtaining quinone from the flour beetle. Ladisch (1953) reported on the carcinogenic effects of quinone contaminants in food products.

In a study investigating the carcinogenic effects of locally applied p-benzoquinone, Tiedemann (1953) did not observe neoplasms in a group of 80 mice repeatedly treated with benzoquinone in a benzene solution. Loconti and Roth (1953) determined the composition of *T. castaneum* secretions. In a series of experiments analyzing the carcinogenic effects of p-benzoquinone, Kishizawa (1954, 1955, 1956) exposed mice to benzoquinone vapor and observed pulmonary carcinoma in several animals. In 1957, Umeda administered injections of p-benzoquinone in a propylene glycol solution to a group of 24 rats. The author observed two cases of fibrosarcoma on experimental days 423 and 481.

Ladisch (1963, 1964, 1965a,b), Ladisch and Suter (1965, 1967), Ladisch and Suter (1963) and Ladisch et al. (1967, 1968) isolated toxic quinones produced by insects to determine their carcinogenicity and the presence of other carcinogens. The secreted substance contained 2-methyl-p-benzoquinone and 2-ethyl--p-benzoquinone at a 1:4 ratio, and it was termed "Tetritoxin". The authors concluded that quinones are a natural carcinogen. Ladisch (1964) suggested a correlation between human neoplasms and milled products contaminated with quinone secretions of *Tribolium* beetles and other insects of the darkling beetle family (Ladisch et al. 1967). In 1968, Ladisch et al. determined 15 cases of sweat gland carcinoma in 41 CBA mice after single exposure to insect quinones, and one sweat gland tumor in the control group of 51 animals.

Shimkin et al. (1971) conducted a 100-week-long experiment on Long-Evans rats which were divided into 10 groups based on sex and the administered substance: MBQ (2-methyl-1,4-benzoquinone), EBQ (2-ethyl-1,4-benzoquinone) and TT (mixture of MBQ and EBQ). The tested substances were administered by s.c. injection in four doses of 3 mg and 16 doses of 6 mg. Injections of identical volume, totaling 108 mg in 7.2 ml, were administered every other week. Tumors were observed in all 10 animal groups, and the first instance of carcinoma was noted after 66 weeks. Four tumors were reported in the group of 31 rats receiving MBQ, seven tumors – in the group of 28 animals treated with EBQ, and five tumors – in 27 rats administered TT. Four neoplastic changes were observed in the control group of 20 animals.

Omaye, Wirtz and Fruin (1981) determined LD_{50} values of 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone administered to male rats in doses of 165, 145 and 205 mg/kg. An analysis of percent lines of the applied doses of p-benzoquinone and its substitutes revealed curves that were not significantly displaced ($p > 0.05$) from parallel lines. 2-methyl-1,4-benzoquinone was 0.4 times more toxic than 2-ethyl-1,4-benzoquinone, and LD_{50} in a single dose of 1-pantadecene was estimated at more than 10 g/kg. Based on subjective results of animal observations, the above authors concluded that quinone exerts a toxic effect by disrupting the functioning of the respiratory system.

El-Mofty et al. (1988) investigated the carcinogenic effect of benzoquinones administered *per os* to *Bufo regularis* tadpoles. The experiment was carried out on mature tadpoles of both sexes, with body weight of around 50 g, divided into two groups (control and experimental) of 100 animals each. Flour (200 mg), colonized by the red flour beetle for 1 year, was mixed with 1 ml of water and administered to control group tadpoles three times a week. The applied dose equaled 1/3 LD_{50}/30 days. The experiment lasted 14 weeks. In the group of 100 experimental animals, 22 tadpoles developed liver tumors, of which six were also diagnosed with liver tumors. Microscopic observations revealed the presence of hepatocellular carcinoma cells in hepatic lobules, whereas secondary foci of primary hepatic tumors were found in the kidneys. Neoplasms were not determined in control group animals.

In a follow-up study, El-Mofty et al. (1989) investigated the effect of infested flour in biscuits fed to tadpoles and biscuit baking temperature on the carcinogenic properties of flour. Similarly to the previous experiment, tadpoles were divided into two groups. Experimental group animals were fed 200 mg ground biscuits mixed with 1 ml water. About 2 kg of biscuit flour had been infested with 20 red beetle individuals for one year. Ca. 22% tadpoles from the experimental

group developed hepatocellular carcinoma metastasizing to the kidneys. Neoplasms were not observed in the control group. The authors of the study concluded that baking temperature did not minimize the carcinogenic effect of flour infested with *T. castaneum* beetles.

El-Mofty et al. (1992) continued their research in an experiment on Swiss albino mice administered flour infested by the red flour beetle, biscuits made of contaminated flour as well as 1,4-benzoquinone. Ca. 35% of the animals fed infested flour developed hepatocellular carcinoma and lympholeukemia. Neoplasms were observed in 29% mice fed biscuits, mostly in the liver (lympholeukemia) and nipple (type A adenocarcinoma). In 33.6% animals administered 1,4-benzoquinone, neoplasms were found in the liver and spleen (lympholeukemia). The researchers suggested that 1,4-benzoquinone, administered alone or in combination with other quinones secreted by beetles into the flour, may be a cause of neoplasms in mouse organs.

Elhassanen and El-Moaty (2003) grew *Tribolium confusum* beetles in wheat flour for eight weeks. Insects were removed from the flour which was fed to rats over a period of 10 weeks. Blood samples were collected every two weeks for the determination of oxidative stress indicators in the plasma and red blood cells. The results obtained pointed to lower activity levels of the antioxidant enzymes GSH-Px and GSH-R in the red blood cells of rats fed contaminated flour. A 9.72% and 32.09% drop was found, respectively, in the activity levels of the enzymes studied. The GSH/GSSG ratio in the plasma, a sensitive indicator of susceptibility to oxidative stress, was much lower at 5.29 ± 1.35. The plasma concentrations of antioxidant vitamins (A, C and E) were also much lower in rats fed contaminated flour, reaching 38.25%, 37.49% and 43.21%, respectively. Plasma TBARS levels, nitrite (N_2) and nitrate (NO_3) concentrations were higher than those determined in the control group by 124.92%, 188.97% and 178.35%, respectively. Elevated TBARS, N_2 and N_3 levels suggest that not only red blood cells, but also other cells and tissues could be exposed to oxidative stress. The results of the study indicate that the administration of infested flour lowers the protective potential of erythrocyte antioxidants and non-enzymatic plasma antioxidants, accompanied by high concentrations of plasma oxidants, including TBARS and nitrogen oxide. Pest-infested flour supports the formation of reactive oxygen species and compounds that enhance the quantity of ROS through redox cycles.

Conclusions

The results of the reviewed studies indicate that benzoquinones secreted by flour beetles may have a toxic effect on humans and animals. This effect may be direct or indirect where, after a series of enzymatic transformations via the metabolic pathways, benzoquinones become secondary metabolites of benzene, suppressing cells and tissues and causing carcinogenic changes. Due to the scarcity of in-depth research investigating carcinogenicity in mammals, follow-up work is required in this area.

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