No Evidence for a Diuretic Effect of Benzisothiazolinone (BIT) in an Experimental Animal Model Employing Anaesthetised Rats

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Abstract: No Evidence for a Diuretic Effect of Benzisothiazolinone (BIT) in an Experimental Animal Model Employing Anaesthetised Rats: Björn Hellman, et al. Department of Occupational and Environmental Medicine, University Hospital, Uppsala—The diuretic effects of benzisothiazolinone (BIT), vapours from a water-based paint, and the reference compound furosemide, were investigated in an experimental model employing anaesthetised rats. BIT (90 mg/kg body weight) was given orally 2 or 12 hr before the rats were anaesthetised. The paint vapours and furosemide were administered during anaesthesia. Urinary flow, osmolality, urinary concentrations of sodium and potassium, and blood pressure were measured as indicators of renal function. Whereas all these parameters were significantly affected after an injection with furosemide, neither BIT nor the vapours from the paint were found to induce any biologically significant effects. Provided that the experimental model with anaesthetised rats is relevant when studying the mechanisms for diuresis in man, the results of the present study suggest that previously reported problems with frequent urination among painters working with water-based paints are symptoms not related to a direct diuretic effect of BIT or the vapours from these paints.

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Key words: Water-based paint, Benzisothiazolinone, BIT, Biocide, Furosemide, Renal function, Experimental model for diuresis, Rat

Several epidemiological studies have shown that occupational exposure to organic solvents may cause acute and chronic dysfunction of the peripheral and central nervous system1). When the association between exposure to organic solvents and CNS effects became more commonly accepted, the paint industry started to develop ‘water-based’ paints (also known as ‘water-borne’ paints) containing considerably smaller amounts of organic solvents than the traditional ‘solvent-based’ paints. The chemistry of water-based paints is more diverse and complex than that of paints more or less entirely based on organic solvents, and the former paints are not completely devoid of organic solvents as indicated by the presence of, for example, glycols, glycol ethers and other volatile organic compounds (VOC’s).

The water-based paints are generally based on synthetic polymers, which means that various additives have to be added in order to improve both the technical quality as well as the longevity of the paint. Consequently, apart from water and organic solvents, water-based paints also contain various biocides, surfactants, pigments, binders, amines and monomers2). The chemical complexity of the water-based paints may introduce new potential health hazards to house painters using great quantities of these paints in their work. Swedish painters have reported skin irritation, stomach problems, and more frequent urination when working with these paints3). An increased excretion of albumin in the urine has also been observed in painters working with water-based paints, indicating a subtle effect on renal function4). There are also other reports available showing a kidney dysfunction in painters occupationally exposed to organic solvents4,5).

Ulfvarson et al.6) published a paper where they reported a temporary increase in urine excretion and a concomitant decrease in urine density among painters exposed to experimental water-based paints. In the latter study it was suggested that the urinary effects could have been caused by benzisothiazolinone (BIT), a biocide present in several water-based paints to protect them from getting damaged by microorganisms. According to the Safety Data Sheet provided by the manufacturer Zeneca Biocides, BIT is a potentially harmful compound with irritant properties. A temporary increase in urine excretion and/or a more frequent urination is not only a comfort
problem for painters occupationally exposed to water-based paints, it may also constitute a potential health hazard. Substantial disturbances in the urinary balance of water and electrolytes can in the long run increase the risk for cardiovascular diseases, and it is known that permanent disability of the kidneys to excrete sodium is associated with increased blood pressure. Using an animal model for diuresis, the following experimental study was undertaken to further elucidate the potential consequences of exposure to BIT and/or water-based paints on the urinary function. If the water-based paints actually do affect the renal function in occupationally exposed individuals, the mechanism behind this effect remains unclear. Moreover, although BIT has been suggested to be a diuretic agent, there are no studies actually showing such an effect of this particular component in the water-based paints.

Materials and Methods

Chemicals: Proxel Press Paste, an aqueous paste containing 90% of the technical quality of 1,2-benzisothiazol-3(2H)-one (BIT, CAS-reg. No. 002634-33-5), and the vehicle hydroxypropyl methycellulose (HPMC) were supplied by Zeneca Biocides, Blackley, Manchester, UK, and Zeneca Pharmaceuticals, Alderly Park, Macclesfield, UK, respectively. A BIT-containing water-based latex emulsion paint for wet rooms ('Resistent Täckfärg,' order No. 011969; Alcro-Beckers AB, Stockholm, Sweden) was purchased in an ordinary hardware store. Furosemide (Benzon Pharma AB, Stockholm, Sweden) was purchased in an ordinary hardware store. Furosemide (Benzon Pharma AB, Copenhagen, Denmark) was used as a reference diuretic, and the anaesthetic agent Inactin® (5-sec-buthyl-5-ethyl-2-thiobarbiturate) was a product of Byk-Gulden, Konstanz, Germany. All other chemicals were of analytical quality. Deionized water was used throughout the experiments.

Animals and surgical procedure: A total of 34 adult male F1 hybrids of Lewis × DA rats from our own breed weighing 250–300 g were divided into six different groups with 4–7 animals in each. The Lewis and DA rats used for the breeding were purchased from ZFK, Hannover, Germany. Before the experiments, the animals had free access to tap water and a standardised rodent chow (R3, Ewos AB, Södertälje, Sweden) containing 3 g sodium, 8 g potassium and 13 megajoule/kg diet.

At the time of the urinary sampling, one rat at a time was anaesthetised by injecting 120 mg Inactin®/kg body weight intraperitoneally. The anaesthetised rat was placed on a servo-controlled heating pad to maintain the rectal temperature close to 37.5°C. After tracheostomy, the left femoral vein was cannulated for continuous infusion of a Ringer solution containing 1.29 mM NaCl, 2.5 mM KCl, 25 mM NaHCO3 and 0.75 mM CaCl2 (5 ml per hour and kg body weight) to compensate for fluid losses during the experiment. With a rubber hose (length: 2 m; diameter: 0.5 mm) with a funnel at the end, humidified air (approximately 150 ml/min of air that had been bubbled through 100 ml water in a gas washing bottle) was applied over the tracheal catheter of the anaesthetised rat. The left femoral artery was cannulated for continuous recording of the blood pressure. The urinary bladder was catheterised through a suprapubic incision for subsequent collection of five 20-min samples of urine after a 1-hr period of recovery after surgery. In the experiments with BIT, the rats were exposed before anaesthesia and surgery. In the experiments with the water-based paint and furosemide, the rats were exposed during anaesthesia. All animals were killed at the end of the experiments.

Experimental design: When investigating the potential diuretic effect of BIT, Proxel Press Paste was suspended in hydroxypropyl methycellulose (HPMC) and given by gavage as a freshly prepared emulsion, either two or twelve hours before the rats were anaesthetised. The amount of Proxel Press Paste administered was 100 mg/kg body weight, which means that each animal received an oral dose of approximately 90 mg BIT/kg b.wt. A third group of rats received an oral dose of HPMC only (0.2 ml per animal), two hours before being anaesthetised. The number of rats in each of these three groups was 7.

When investigating the potential diuretic effect of the water-based paint containing BIT, a group of four anaesthetised rats were exposed to a mixture of humidified air and paint (vapours rather than an aerosol, see below) which was led from the gas washing bottle through the rubber hose to the funnel applied over the tracheal catheter. The mixture of humidified air and paint was generated by adding 100 ml of water-based paint to 100 ml of water in the gas-washing bottle (see above). The exposure began 40 min after beginning with the urinary sampling and continued for 1 hr with uninterrupted sampling of 20-min portions of urine. Another group of rats (4 controls) were exposed under similar conditions to humidified air only. To ensure that a diuretic effect could be detected under the experimental conditions employed, another group of 5 anaesthetised rats exposed to humidified air only were given an intravenous injection of furosemide (400 µg/kg body weight) after 40 min of urinary sampling.

Urinary measurements: From each animal, five subsequent 20-min samples of urine were collected into pre-weighted plastic vials. In the experiments with BIT and the vehicle HPMC, all these samples represented urine from exposed animals. Since the first two urinary samples were taken before exposure in the experiments with the water-based paint, and furosemide, only the three last represented urine from exposed animals. Following previously described procedures, the following parameters were recorded from each individual animal: (i) urinary flow, (ii) osmolality, (iii) urinary concentrations of sodium and potassium, and (iv) blood pressure.
**Estimation of VOC concentrations in the mixture of humidified air and paint:** The exposure conditions in the experiments with the water-based paint were characterised by sampling volatile organic compounds for 60 min at the end of the rubber hose (i.e., in the funnel region) under continuous bubbling of air through the gas washing bottle (150 ml/min) containing 100 ml water and 100 ml paint. VOC's were sampled with two different sorbent tubes (XAD-7 and SKC 226-94; SKC Inc., Pa, USA) connected to a battery-operated pump, at a pump flow rate of 130 ml/min. No animals were present during the sampling. Compounds adsorbed on the synthetic polymer sorbent XAD-7 were desorbed with dichloromethane, and those adsorbed on the charcoal sorbent SKC 226-94 were extracted with carbon disulphide. Analysis was performed by means of gas chromatography (Hewlett Packard, 5890 series II; USA) equipped with a mass selective detector (Hewlett Packard, model 5971A) and a DB-5 column (60 m, 0.32 mm, 1.0 µm; J&W Scientific, Folsom, Ca, USA). The following oven temperature programme was used during the analysis: 35°C for 5 min, 22°C/min up to 200°C, 220°C for 5 min, 20°C/min up to 270°C and then 270°C for 10 min.

**Statistical evaluation of data:** A two-way analysis of variance with repeated measures in the time parameter were used for the between group comparisons. Contrasts were used for comparisons within the groups: control period (periods 1 and 2) against period 3 or periods 4 and 5. The t values were Bonferroni adjusted. When assessing the potential effect of the solvent vapour on the urine osmolality, this parameter was also analysed with Tukey's HSD-test at different time periods (between groups).

**Results**

As indicated in Fig. 1, oral administration of BIT, in the form of 100 mg Proxel Press Paste/kg body weight two or twelve hours before the animals were anaesthetised, did not significantly affect the various parameters for renal function. The urinary concentrations of sodium and potassium were slightly decreased in the rats given BIT during the first 40 min of urinary sampling (Fig. 1A), but these differences were only marginal in comparison to the vehicle-treated control animals and well within the normal variation for anaesthetised animals. The same was true for the slight increase in urinary flow (Fig. 1C), and decrease in osmolality (Fig. 1D) observed during the last 20 min of sampling in animals given BIT 12 hr before surgery. As indicated in Fig. 2, BIT did not affect the blood pressure.

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**Fig. 1.** The renal excretion rates (mean ± SEM) of sodium ($U_{NaV}$) and potassium ($U_{Kv}$), urine flow rate and osmolality of rats fed BIT 2 or 12 hr prior to anesthesia or vehicle treatment. Stars indicate a significant difference ($p<0.05$) between periods 1 and 2, and periods 4 and 5 (within group comparisons). There was no difference between the different groups ($p>0.05$).

**Fig. 2.** The mean arterial blood pressure (MAP) of rats fed BIT 2 or 12 hr prior to anesthesia or vehicle treatment. Stars indicate a significant difference ($p<0.05$) between periods 1 and 2 and periods 4 and 5 (within group comparisons). There was no difference between the different groups ($p>0.05$).
Figure 3 presents the results from the experiments with the reference compound furosemide and the water-based paint containing BIT. Furosemide (400 µg/kg b.wt) showed all the effects that could be expected from a classical diuretic agent with an instant action. Immediately after the injection of furosemide (i.e., 40 min after beginning the collection of urine), the excreted amounts of both sodium (Fig. 2A) and potassium (Fig. 2B) were significantly increased (P<0.001), and so was the urinary flow (Fig. 2C). The osmolality was significantly reduced (P<0.001) shortly after the injection (Fig. 2D).

The exposure analysis, with volatile organic compounds as exposure indicators, showed that the rats were exposed to compounds in the paint in the experimental set-up with air bubbling through the mixture of paint and water in a gas washing bottle. The following major VOC’s were identified: Hexadecane (13.0 mg/m³), 2-butanol (6.4 mg/m³), 1-butanol (0.4 mg/m³), 1,3-diethylbenzene (0.4 mg/m³), undecane (0.4 mg/m³), 1,3-diethylbenzene (0.4 mg/m³) and toluene (0.15 mg/m³).

In contrast to the highly significant effects induced by furosemide, the vapours from the water-based paint were found to be without significant effects on the concentrations of electrolytes (Fig. 3A and B), and urinary flow (Fig. 3C). The osmolality was decreased among the rats inhaling the paint vapours, but this decrease was probably not related to the exposure since this parameter was found to be decreased also before the animals were exposed to the paint vapours (Fig. 3D).

There was a slight decline in blood pressure with time in all groups studied, but this decline was not affected by treatment. Consequently, as indicated in Fig. 4 also furosemide and the paint vapours were found to be without significant effects on blood pressure.

Discussion

Problems with frequent urination have been reported among painters working with water-based paints. The present study showed that neither the oral administration of a high dose BIT, nor the acute inhalation of vapours from a water-based latex emulsion paint for wet rooms affected the renal function in an experimental animal model for diuresis. In the same experimental model, furosemide was found to exert significant effects on all the investigated renal parameters.

The paint used in the present study was an emulsion paint containing mostly water, acrylate co-polymers and
titanium oxide. According to the manufacturer, these three components constituted approximately 45, 25 and 20% of the total content in the paint. The stated concentration of BIT was 0.01% (w/v). Although the paint apparently did contain BIT, it is not known if the rats actually were exposed to this compound in the experiments with the water-based paint. BIT, a compound with low vapour pressure not likely to escape easily from the paint to the surrounding air, was not included among the compounds measured when characterising the exposure situation for the anaesthetised rats. Nevertheless, the exposure measurements showed that the rats were exposed to a complex mixture of volatile organic compounds in concentrations similar to those encountered by painters working with water-based paints. Interestingly, several of the identified VOCs in the exposure assessment (e.g., hexadecane, 1,3-diethylbenzene and toluene) were not included among the agents mentioned in the manufacturer’s declaration of contents. The reason for this discrepancy is not yet known.

The rats given an oral dose of Proxel Press Paste were indeed exposed to BIT. The exact dose is not known, but depending on the actual content in the paste, the administered dose of BIT was somewhere in the range of 60 to 90 mg/kg body weight. This represents an enormous dose of BIT, at least in comparison to the dose painters can be expected to be exposed to during the occupational handling of water-based paints. Painters are most likely exposed to BIT either by direct skin contact with the paint, or via inhalation of an aerosol in the paint, but the present study was performed on rats given the compound orally. This route of administration was chosen to ensure that the animals actually received the compound. Injection was not possible due to the solubility problems with the Proxel Press Paste.

Under the experimental conditions employed, both BIT and the vapours from the water-based paint were found to be without significant effects on urinary flow, osmolality, urinary concentrations of sodium and potassium, or blood pressure. The absence of significant effects indicates that the previously reported problem of frequent urination among painters working with water-based paints is probably not related to a true diuretic effect of the water-based paint. The rats were exposed to relatively high concentrations of volatile organic compounds and, although the exposure time was limited (1 hr), it should have been possible to detect a diuretic effect, especially since the reported urinary problems among the painters seem to be an acute effect. Unless the problems with frequent urination among painters working with water-based paints are due to effects that can be suppressed during anaesthesia, other explanations than imbalances in electrolytes and/or urinary water content must be sought in order to explain the reported renal problems of the painters.

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