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FIELD-WATER QUALITY STANDARDS FOR BZ

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Winifred G. Palmer

January 1990

U S ARMY BIOMEDICAL RESEARCH & DEVELOPMENT LABORATORY
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Effects of BZ in animals and humans were evaluated and served as the basis for the development of a water quality standard for field drinking water. BZ can reduce mental and physical performance at very low levels and hence is a substantial concern in drinking water. The 7-day standards for daily consumption of 5 and 15 liter water are 7 ug/liter and 2.33 ug/liter, respectively. Due to insufficient data in animals or man, a long-term drinking water standard (>7 days to ≤1 year) could not be developed.

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TABLE OF CONTENTS

INTRODUCTION 1

BIOCHEMISTRY 1

PHARMACOKINETICS 1

ANTIDOTES 2

CHEMISTRY 2

- Behavior in Water 2
- Detection in Urine 3
- Detection in Water 3
- Water Purification 6
- Hydrolysis Products and Storage Stability 6

EFFECTS IN HUMANS 7

- Signs and Symptoms 7
- Acute Exposures 7
- Long-term Exposures 11

CARCINOGENICITY 12

REPRODUCTIVE SYSTEM 12

MUTAGENICITY 12

EFFECTS IN ANIMALS 12

- Short-term Exposures 12
- Effects on Behavior 15
- Multiple Exposures 17

CONCLUSION 19

REFERENCES 23

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Availability Codes
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LIST OF FIGURES

1. Structure of BZ and its hydrolysis products 4

2. Rate constant versus pH profile for BZ hydrolysis, 25°C 5

3. Health-effects summary for BZ 22

LIST OF TABLES

1. Variation of hydrolysis rates with pH and temperature 3

2. Toxicity of BZ hydrolysis products in the mouse 6

3. Approximate time of onset and duration of physiological and psychological changes resulting from a single intravenous dose of 8 μg/kg in human subjects. 8

4. Effects in man of single i.m. or i.v. exposures to BZ 10

5. Effects of multiple daily exposures to BZ 13

6. Effects of intravenous BZ in various animal species 14

7. Effect of single i.v. injections of BZ in the dog 15

8. The effects of BZ on behavior 16

9. Effects of 6-week exposures to BZ 18

INTRODUCTION

BZ (3-quinuclidinyl benzilate) is a potent muscarinic cholinergic antagonist. Small doses can affect the brain and nervous system producing incapacitation. Although BZ is considerably more potent, its toxic signs are similar to those of atropine, one of the most widely studied of the antimuscarinic agents. These drugs inhibit the action of acetylcholine on autonomic effectors innervated by postganglionic cholinergic nerves as well as on smooth muscles that lack cholinergic innervation. The effects of the antimuscarinic drugs are qualitatively similar, but individual drugs in this class of compounds differ quantitatively in their actions on the various systems vulnerable to their effects. Typical of this class of drugs, in small doses BZ depresses salivary secretion and sweating; while with larger doses the pupil dilates (mydriasis), accommodation of the eye is inhibited (cycloplegia), and vagal effects on the heart are blocked, increasing the heart rate. Still larger doses inhibit parasympathetic control of the gastrointestinal tract, decreasing the tone and motility of the gut. Other effects include weakness, ataxia, prostration, hallucinations, delirium, and death [1].

At higher doses, antimuscarinic drugs may block or reduce responses to neurotransmitters other than acetylcholine, causing effects that are not related to their antimuscarinic activity. The hallucinogenic effects of BZ are believed to be due to binding to a receptor subtype specific for the neurotransmitter serotonin in serotonin target neurons [2].

BIOCHEMISTRY

Presynaptic autoreceptors, which modulate the release and synthesis of neurotransmitters during nerve stimulation, have been identified in rodent brains [3]. Presumably by interacting with these receptors, BZ and other muscarinic receptor antagonists stimulate the in vitro release and synthesis of acetylcholine by rat brain preparations [3,4]. Marek found that while BZ significantly increased acetylcholine synthesis in human brain, it was far more effective in rat brain. Of 20 antimuscarinic agents tested, BZ was the most active [3].

Another action on brain tissue is the BZ-induced block of the elevated respiration and glycolysis that normally accompanies electrical stimulation of rat cerebral cortex and medulla oblongata; respiration in unstimulated tissue is not affected by BZ [5]. Also, BZ can cause biochemical changes in other tissues. It is a potent inhibitor of Ca^{++} uptake by rat heart mitochondria, but it does not alter the rate of binding of Ca^{++} in rabbit skeletal muscle microsomes [6]. BZ caused a tenfold greater inhibition of pancreatic amylase secretion than did atropine [7] and was more active in inhibiting acetylcholine-induced spasms in isolated strips of guinea pig ileum [8].

PHARMACOKINETICS

Little information is available on the metabolism, distribution or elimination of BZ beyond that reported in 1963 by Zvirblis et al. [9]. That study showed that nearly all the tritiated BZ injected intraperitoneally

(i.p.) into female albino rats was present in urine and feces within 24 hours. Only about 3 percent of the excreted radiolabel was present as unmetabolized BZ, and this was largely excreted within the first few hours after injection. The metabolite 3-quinuclidinol was identified in urine by paper chromatography. Elimination through the intestinal tract occurred more slowly and about 15 percent of the injected radiolabel remained in the cecum 2 days after administration.

Relatively high levels of radiolabel appeared briefly in the lung and spleen; radiolabel remained in the kidney and liver for longer periods of time. The majority of the label in the liver and plasma was identified as BZ metabolites.

ANTIDOTES

Physostigmine salicylate (Eserine) is a highly effective treatment for BZ poisoning [10]. It can be administered by injection or ingestion; frequent dosing is necessary. Physostigmine can reverse the delirium induced by BZ and will enable individuals to achieve normal behavior patterns in about 8 hours. With the major exception of visual difficulties, most of the physiological symptoms (e.g., elevated heart rate, dry mouth, anorexia) can be reversed by Physostigmine.

CHEMISTRY

The molecular structure of BZ and its hydrolysis products benzilic acid and 3-quinuclidinol are shown in Figure 1.

Behavior in Water

The solubility of the free base in water is low; its maximum solubility in water at 25°C is 11.8 mg/l [11]. In contrast, salts of BZ are generally quite water-soluble. The solubility of BZ can be expressed as follows [11]:

$$[\text{total BZ}]_{max} = \frac{([H^+] + K_a)\,[\text{BZ free base}]_{max}}{K_a}$$

The solubility of BZ increases with the hydrogen ion concentration. The pH also influences the rate of hydrolysis, which is most rapid in very basic solutions. In less basic solutions, the hydrolysis occurs slowly and is influenced by factors such as solubility. In addition, the solubility of the ionized form of BZ can be greatly influenced by the nature of the counter ion. This has not been addressed in the literature. The relationship between the hydrolysis reaction rate and pH are shown in Figure 2.

Table 1 shows the effect of pH on the half-life of BZ in basic solutions. The half-life increases from 0.3 hours to 6.7 hours as the pH is reduced from 13.7 to 9.8. It is clear from Figure 2 that the half-life increases markedly as the pH is further reduced. In the pH range of drinking water, the solubility of BZ and the rate of hydrolysis are such that sufficient levels of BZ could be attained in drinking water to present a threat to health.

TABLE 1

VARIATION OF HYDROLYSIS RATES WITH PH AND TEMPERATURE

Temperature (°C)	pH	Half-life (hrs)
25-27	9.8	6.67
25-27	10.8	1.83
25-27	12.2	0.83
25-27	13.7	0.30
37	7.0	200.0
37	7.4	95.0
37	8.3	30.0
37	9.0	9.5
37	10.0	2.9
37	11.0	0.8
37	12.0	0.2
37	13.0	0.06

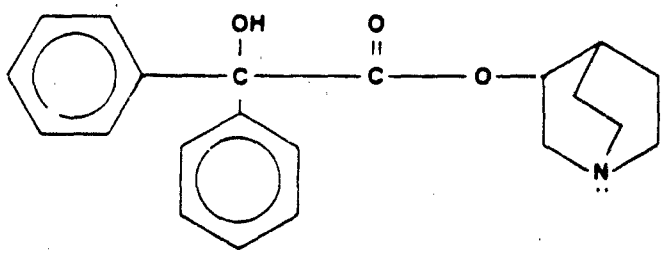
Data from work of Sass et al. [12,13]

Detection in Urine

Byrd et al. [14] developed an analytical procedure using isotope dilution gas chromatography/mass spectrometry for determining concentrations of BZ and its major metabolites in urine. In this procedure, beta-glucuronidase is added to urine samples to hydrolyze conjugated BZ. The detection limits are better than 0.5 ng/ml for BZ and 5 ng/ml for the metabolites benzilic acid and 3-quinuclidinol. The lower detection limits were designed with the assumption that in the first 500 ml of urine from a 70 kg man, 2 percent of the absorbed compound would appear as unmetabolized BZ while 40 percent of the original dose would be present as its primary hydrolysis products benzilic acid and 3-quinuclidinol. The available data do not permit estimation of actual exposures from the concentrations of BZ or its metabolites in urine or other body fluids.

Detection in Water

Analytical methods for BZ were reviewed by Rosenblatt et al. in 1977 (11). The methods discussed include electrophoresis, qualitative color tests, thin-layer chromatography, photometric methods, and gas-liquid chromatography. In 1987, Byrd et al [14] reviewed published methods for the analysis of low concentrations of BZ. Several methods using GC/MS or high pressure liquid chromatography were briefly described for the analysis of low concentrations of BZ in aqueous solutions. At least one GC method had a sensitivity in the range of 1 to 10 μg/ml.



BZ (3-Quinuclidinyl Benzilate)

Hydrolysis Products of BZ

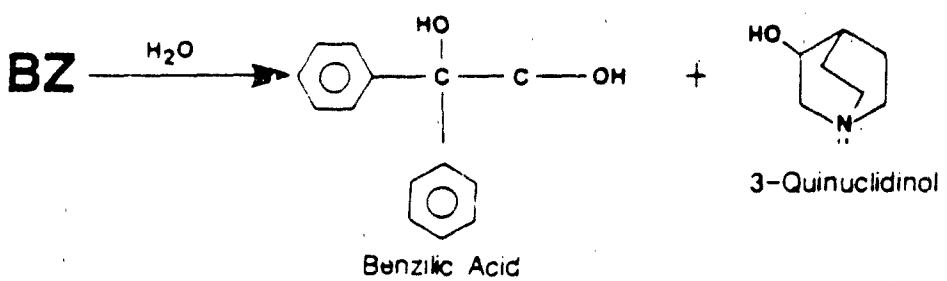


Figure 1. Structure of BZ and its hydrolysis products. From Rosenblatt *et al.*[11]

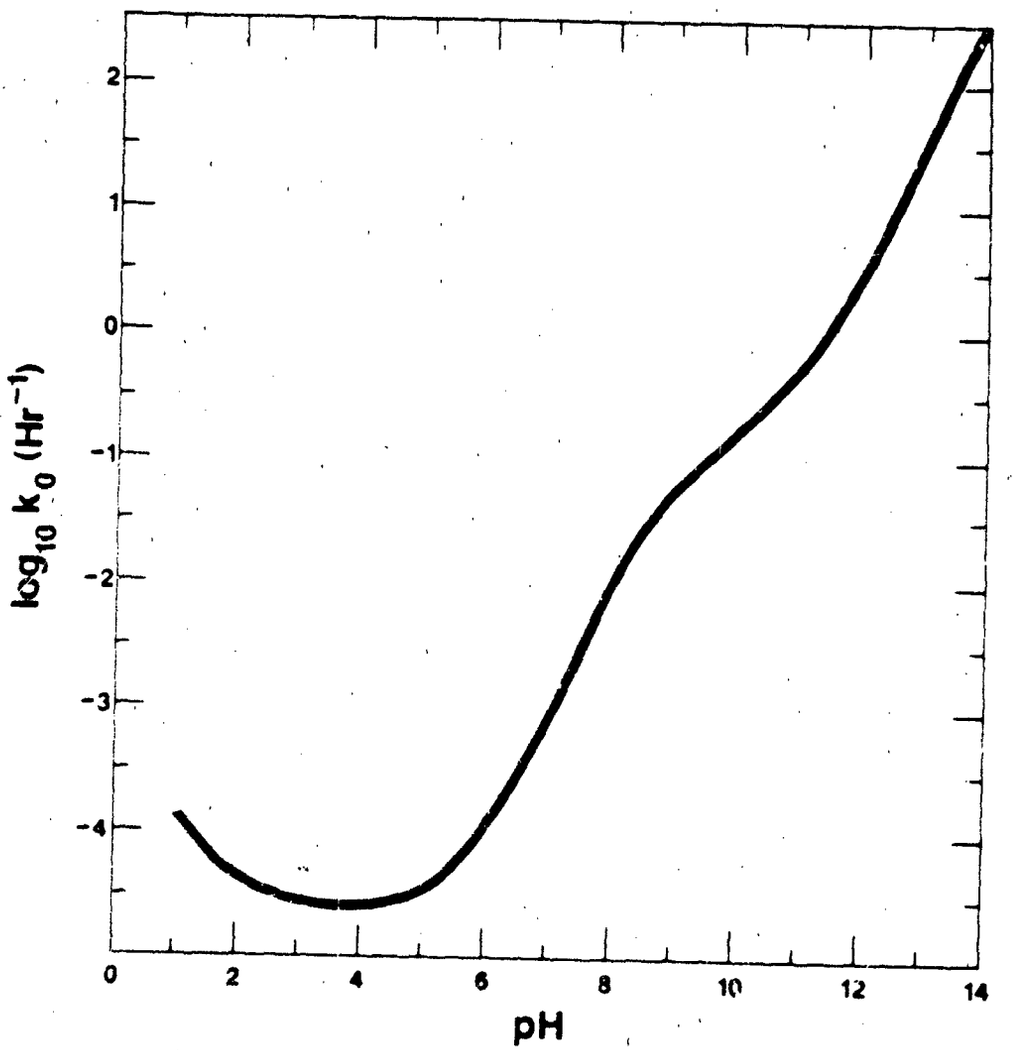


Figure 2. Rate Constant vs pH Profile for BZ Hydrolysis, 25°C. From Rosenblatt et al.[11]

A technique for detecting BZ in aqueous solutions in the field with a sensitivity of 5 μg using a Marquis reagent [15] was mentioned in Byrd's review. Insufficient information was presented to enable an evaluation of the applicability of this technique to the monitoring of drinking water. According to Eskelund, [16] analytical units for monitoring BZ levels in water for use in the field during military operations have not been successfully developed.

Water Purification

The capability of the 600 GPH ROWPU water purifying equipment for field decontamination of water containing chemical contaminants was tested at Aberdeen Proving Ground in 1975 (19). This system could effectively reduce BZ concentrations in water from 6.96 mg/l to 0.005 mg/l.

Hydrolysis Products and Storage Stability

McNamara [17] compared the relative toxicities of some hydrolysis and pyrolysis products of BZ (Table 2) by determining LD50s following intravenous (i.v.) injection in the mouse. Only one of the compounds tested (benzhydrol) had an LD50 below the 18 to 25 mg/kg LD50 range that has been reported for BZ in the mouse. These limited data suggest that the hydrolysis products quinuclidinol and benzilic acid should be less toxic than the parent compound.

TABLE 2

TOXICITY OF BZ HYDROLYSIS PRODUCTS IN THE MOUSE

Compound	LD50 (mg/kg)
Methyl benzilate	25.6
Benzhydrol	17.4
Benzophenone	28.9
Quinuclidinol	179
Benzilic Acid	>400
BZ	18 - 25

Data from McNamara [17]

According to Rosenblatt et al., BZ is considerably more toxic than its hydrolysis products (11). However, they cited no relevant data. The current review revealed no data on the relative toxicities of hydrolysis products in nonlethal doses.

In a similar experiment, McNamara used LD50s determined by intraperitoneal (i.p.) injection in the mouse to evaluate the stability of samples stored in glass containers at temperatures of 70 to 90°F [17]. BZ hydrochloride was

more stable than the free base. The LD50s of BZ hydrochloride did not change substantially for up to 14 weeks. Several solvents (i.e., 0.1 N HCl, polyethylene glycol, ethyl alcohol, propylene glycol) were tested and none altered the stability. In contrast, BZ in the form of the free base deteriorated with time when it was dissolved in 30 percent ethanol or propylene glycol.

EFFECTS IN HUMANS

Signs and Symptoms

BZ intoxication is manifested by a complex of rapidly progressing symptoms which were described by Ketchum [18] in the following way. Peripheral autonomic effects are characterized by elevated heart rate and blood pressure, increased cutaneous blood flow (facial flushing and elevated skin temperature), dry mouth, and anorexia. Hypoactive peristaltic sounds are evident in many subjects. Exposed persons frequently complain of "weakness" or "tightness" in the legs. An early and common symptom is pupillary dilation with complaints of blurred vision and difficulty in focusing on near objects. The latter response occurs early and persists after recovery from most other effects.

The first neurological signs are increased deep tendon reflexes of the lower extremities, followed by ankle clonus and progressive deterioration of the normal gait; complaints of uncomfortable paresthesias of the lower extremities characterized as aching, tingling, tight feeling, and heaviness without a measurable change in muscle tone or dystonic movements. Tremors of the lips and arms as well as facial muscle twitches may occur at higher doses. Difficulties with speech are a prominent feature. Higher doses cause severe mental depression with the appearance of heavy sedation and confusion.

With increasing doses, the time to onset of symptoms may shorten and their severity intensify. As this happens, the period of maximal incapacitation and the time to complete recovery are lengthened correspondingly [26]. With low doses, symptoms may last for several days. Recovery from severe exposures may take several weeks. The entire complex of BZ symptoms occurs with i.v. doses of about 6 to 8 μg/kg. The order of appearance of symptoms and their duration are shown in Table 3.

Acute Exposures

In 1950, a chemist working at Hoffman La Roche ingested 0.5 mg BZ (approximately 7 μg/kg, assuming a weight of 70 kg) [20]. Shortly thereafter, he noted signs of mydriasis, dryness of the throat and mouth; and he felt weak at the knees. He was disoriented, anorexic, and his speech was incoherent. He later reported having vivid dreams and rousing several times feeling jumpy and anxious during the first night after ingestion of BZ. He was unable to read for several days due to cycloplegia. On the second day, he was still unsteady on his feet, and his mouth felt dry. By the fourth day, the only remaining symptoms were slight dilation of the pupils and some fatigue.

TABLE 3

APPROXIMATE TIME OF ONSET AND DURATION OF PHYSIOLOGICAL AND PSYCHOLOGICAL CHANGES RESULTING FROM A SINGLE INTRAVENOUS DOSE OF 8 μg/KG IN HUMAN SUBJECTS

Symptom	Initial appearance (hours)	Recovery (hours)
Rapid pulse	0.5	40
Dry mouth	1	72
Blurred near vision	1	72
Problem solving*	1	72
Poor coordination	2	36
Restless activity**	3	49
Maximum incapacitation	5	39
Stupor	6	18
Delirium***	8	50

Data taken from Sim [19]

* Inability to solve problems or remember information.
** Restless activity declines during period when stupor is prevalent.
*** Confusion, incoherence, hallucinations, disorientation.

Cummings and Craig [21] examined 14 men subjected to doses of 4 or 5 μg/kg body weight in 250 ml drinking water. Body and skin temperatures were measured during 6 hours of exercise in environments of 85 to 115°F. Symptoms of dry mouth and limb fatigue were present in all participants within 90 minutes to 3 hours after dosing. Fatigue was experienced by 11 men, and tachycardia was present in 8 of the 14 subjects within 2 to 2-1/2 hours. Heart rates were elevated by about 20 percent during walking. After 2 hours, BZ reduced sweating by an average of approximately 30 percent, while skin temperatures were elevated by 2 to 3°F and average body temperatures were 0.5 to 0.7°F above controls. Most men receiving doses of 5 μg/kg became ataxic within 4 hours after exposure. Seven of the 14 men became incapacitated (i.e., they were unable to perform physical work or continue exercising). Two men suffered mild to severe hallucinations and had decreased mental capability. These effects lasted for up to 3 days. It was concluded that during exercise, doses of 4 and 5 μg/kg could induce heat casualties at environmental temperatures of 85°F or higher.

Craig later reported that intramuscular (i.m.) doses of 2 to 6 μg/kg at 41°C (106°F) caused increased heart rate and elevated skin and rectal temperatures which reached a maximum 6 hours after treatment and returned to normal within 24 hours [22]. The disturbances of body temperature at these dose levels were not considered to be hazardous to persons in good health.

Only one study was found which tested for lasting effects after substantial time had passed following exposure to BZ. During a followup study of 31 men who had participated in a drug testing program an average of 20 months earlier, Klapper, et al. [23] examined two men who had received BZ. Neither changes in blood and urine chemistries nor physical or psychological changes could be attributed tc their prior BZ exposure. For the purposes of the current analysis, the results of this study are clearly limited by the number of BZ-exposed participants.

In 1965, Kitzes and Vancil [24] conducted a study to determine the minimal doses of BZ that affect mental and physical functions. The MED50 for mental function was considered to be the dose that caused a 25 percent or greater decrement in the Number Facility Test (the NF test is a speed accuracy test which measures cognitive function). The 13 subjects were given single i.m. doses of 2.3 or 2.7 μg/kg (calculated as the free base).

Drowsiness, blurred vision, and dry mouth were the most common complaints among all subjects. Some exhibited the more severe symptoms of restlessness, inability to sleep, anorexia, and neurological changes. One subject who received 2.7 μg/kg became delirious. Although his response was not severe, this reaction had not been previously observed in men receiving less than 4 μg/kg.

The effect of BZ on performance in the NF test was greatest at 5 hours after dosing. The number of subjects who exceeded the 25 percent decrement on the NF test was 1 of 6 at a dose of 2.3 μg/kg and 5 of 7 at 2.7 μg/kg. From these results, the MED50 for mental function was calculated to be 2.54 μg/kg with 95 percent confidence limits of 2.31 to 2.80.

Physiological effects (dilated pupils but no tachycardia) were observed in three of the six men who received 2.3 μg/kg. Of the seven subjects who received the higher dose, three had dilated pupils, two had tachycardia, and there was one case of hypertension. Based on these data, the MED50 of BZ for pysiological signs was estimated to be 2.7 μg/kg. This is in agreement with Ketchum's earlier estimate of 3 μg/kg for the minimal effective dose [18].

Based on single human exposures by oral, i.m. and i.v. routes, Ketchum [26] calculated the effective doses necessary to produce signs and symptoms of mild, moderate, and severe incapacitation (see Table 4). Mild incapacitation was defined as mydriasis, dryness of the buccal mucosa, and minimal reduction of performance while severely incapacitated persons exhibited frank signs of toxic psychosis. The degree of incapacitation associated with each category were described by Ketchum as follows [26]:

TABLE 4

EFFECTS IN MAN OF SINGLE I.M. or I.V. EXPOSURES TO BZ

Dose (μg/kg)	Effect	Reference
2.0	ED1 (mild)	25
2.3	Dilated pupils (3 of 6 subjects)	24*
2.54	Mental performance, MED50	24*
2.6	ED1 (moderate)	25
2.7	Physiological signs, MED50 (tachycardia, mydriasis)	24*
2.7	Delirium (1 of 7 subjects)	24*
2.9	ED1 (severe)	25
3	Physiological signs, MED	26
4	Tachycardia consistently observed	26
4.4	ED50 (mild)	25
5.1	ED50 (moderate)	25
6.2	ED50 (severe - delirium and incapacitation)	26
4400	LD50 (calculated)	27
5600	LD50 (calculated)	19

* dose given in terms of the free base

Mild - Peak heart rates of 80 to 85 with a blood pressure increase less than 10 millimeters Hg, systolic; moderate dilation of pupils and slight blurring of vision; sleepiness, slight dryness of mouth, minimal incoordination, some mental slowing. No loss of contact with reality. Full recovery time - 50 to 60 hours.

Moderate - Peak heart rates of 85 to 95, with a blood pressure increase less than 20 millimeters Hg, systolic. Symptoms generally the same as for mild effects with increased severity. Fleeting illusions and hallucinations may occur, with brief lapses in ability to concentrate and transient confusion. Full recovery time - 70 to 80 hours.

Severe - Subjects reach very low level of performance. Hallucinations, confusion, hyperactive disorganized behavior, incoherent speech, and disturbances in mentality and attention characteristically appear following an early period of deep sleep or stupor. Peak heart rates reach 95 to 119. Full recovery time - 100 to 140 hours.

Oral exposures are effective in man at doses between 3 and 7 μg/kg [20]. Mild incapacitation has been observed with single oral doses of 5 μg/kg in most subjects tested [25]. Although data are not available to precisely define equivalent i.v. and oral doses, the relative effectiveness of the oral

route has been estimated to be between 75 [25] and 90 percent [26] that of the i.v. route of administration. Intravenous and i.m. BZ doses produce almost identical effects [26].

Long-term Exposures

A repeated dose study was conducted in which groups of men were given i.m. BZ doses of 0.5, 1.0, and 2.0 μg/kg for up to 7 days. This study was described in reference 25 (author unknown) and by Ketchum in reference 26. Both authors concluded that consecutive daily doses of 0.5 μg/kg were tolerated with no signs or symptoms of BZ exposure and that there was little evidence for the development of tolerance to higher doses. However, there were some discrepancies in the severity of effects described for the 1 μg/kg doses. According to the report by Ketchum [26], only minimal effects were seen with successive doses of 1 μg/kg. However, no data were given in reference 25 to substantiate that conclusion. More details and some data were given concerning the reaction of four of the subjects to repeated doses of 1 μg/kg. (Unpublished materials provided by Ketchum were given as the data source in reference 25.)

In that report, these subjects were described as experiencing mild incapacitation after 3 days of dosing. The description of this study given below represents a best effort to resolve the differences between the two reports in the absence of original source materials. To err on the safe side, more emphasis is placed on the data given in reference 25 in determining the maximum safe dose.

Four subjects received 2 μg/kg BZ once daily for 3 days. They were only slightly impaired on the first day but experienced mydriasis and dryness of the oropharyngeal mucosa by the second day. One man was considered to be mildly incapacitated at this time. Moderate to severe effects were noted in three subjects by day 3 and frank signs of toxic psychosis (confusion, disorientation, and other symptoms of delirium) were present in the fourth subject. There was no evidence of tolerance. All subjects were completely recovered within 48 hours after the final dose.

Three subjects were given daily doses of 1.0 μg/kg for 5 days. There were no significant BZ effects during the first 3 days. Early signs of intoxication, including mydriasis, dryness of the buccal mucosa, and minimal reduction of performance were noted on day 4. By day 5, two of the subjects were mildly incapacitated [25].

Four subjects received 1.0 μg/kg i.m. daily for 3 days. Minimal signs and symptoms were observed daily but no signs of a cumulative effect were observed [26].

Four men were given 0.5 μg/kg i.m. daily for 6 days over an 8-day period. (Dosing was conducted on days 1-5 and day 8.) No signs suggestive of BZ intoxication were noted.

It can be concluded from this study that BZ has a cumulative effect at doses of 1 and 2 μg/kg but not 0.5 μg/kg. Tolerance to consecutive doses of BZ was not evident in most subjects. Repeated doses of 0.5 μg/kg was endured for a short period with no signs or symptoms of BZ exposure.

In a second repeated dose study, seven men were exposed by inhalation to an amount of BZ equivalent to an i.v. dose of about 6 μg/kg. Two to 3 weeks later, they received an i.m. dose of 6 μg/kg. Six of the subjects exhibited an enhanced reaction to the second exposure; the symptoms developed more rapidly and were more severe than would be expected with a single dose of 6 μg/kg. Their recovery was accelerated during the first 10 to 15 hours after the second exposure. However, the total recovery time was not appreciably shortened [26].

CARCINOGENICITY

No studies of the carcinogenicity of BZ have been conducted.

REPRODUCTIVE SYSTEM

When introduced through a window in the egg shell, BZ did not affect chick embryo development. Parenteral administration of 1 mg/kg had no effect on fertility or fetal development in mouse multigenerational studies. Subcutaneous injection of BZ (1 mg/kg daily for 10 days) had no effects on fertility or embryo implantation in the mouse [28]. However, in other studies BZ was found to inhibit the fertilization of mouse eggs in vitro by blocking penetration of the zonae pellucidae by spermatozoa [29].

MUTAGENICITY

Intraperitoneal injection of 0.1 to 60 mg/kg did not cause chromosomal aberrations in Chinese hamster bone marrow cells [30]. In addition, BZ was not found to be mutagenic in a number of test systems in Saccharomyces, mice and humans. Sram et al. concluded that, for use in clinical trials, doses of 0.05 to 0.1 mg/kg (with a maximum of 0.5 mg/kg) should not present a serious genetic risk for man [31].

EFFECTS IN ANIMALS

Short-term Exposures

BZ has essentially the same effects in animals and man. However, there are substantial differences among the species in terms of resistance and sensitivity to the compound. In general, increased heart rate, some electrocardiographic changes, mydriasis, cycloplegia, impaired physical performance, and mental effects occur most frequently between 5 to 50 μg/kg; while prostration, convulsion, and death occur in most species between doses of 1 to 10 mg/kg (Table 5).

TABLE 5

EFFECTS OF MULTIPLE DAILY EXPOSURES TO BZ

number of subjects	daily dose (μg/kg)	number of doses	Results
4	0.5	6*	Fatigue, general mild malaise by day 7. No definite signs of BZ effect
4	1.0	3	Minimal effects daily. No evidence of cumulative effects or tolerance.
3	1.0	5	Minimal effects daily.** Possible cumulative effects.***
4	2.0	3	Minimal effects first day. Mild to moderate effects second day. Moderate to severe effected third day. No evidence of tolerance.

* Six doses were delivered over an 8-day period.
** Reference 26.
*** Reference 25.

In both humans and animals, there is little difference between the effects produced by i.m. and i.v. injection. However, unlike humans, in which BZ is only slightly less effective by the oral route than by i.v. or i.m. injection [26], a wide variance in the effects of oral and i.v. doses has been observed in animals. In the dog, there is a 10-fold difference between the oral and i.v. doses that produce mydriasis and as much as a 40-fold difference in the oral and i.v. doses that interfere with sustained physical activity or the conditioned avoidance response (CAR) [17]. The ratio of effective oral to i.v. doses ranges from 25 to <250 for various symptoms in the rabbit. Because of data such as these, relationships between oral and i.v. doses in animals are generally thought to be much less reliable than those found in human studies which severely limits the utility of data from oral dosing of animals in the development of maximum permissible concentrations (MPCs).

LD50s have been determined in several species (Table 6). With the exception of the mouse, whose LD50 is generally reported to be 20 to 25 mg/kg, i.v. injection of BZ generally produces LD50s within the relatively narrow dose range of 5 to 10 mg/kg. Oral LD50s tend to be substantially higher than those produced by i.v. injection and values of 80, 240, and 300 mg/kg were reported for the cat, mouse, and rabbit, respectively [8,11].

TABLE 6

EFFECTS OF INTRAVENOUS BZ IN VARIOUS ANIMAL SPECIES

Dose (mg/kg)	Species	Effect	Reference
0.005-0.01	Dog, rabbit	Increased heart rate	17
0.007	Dog	Ataxia, RD50 (RD1 = 0.003)	19
0.008	Rabbit	Mydriasis, MED	8
0.010	Dog	Marked increase in pulse rate	17
0.010-0.015	Dog	Transitory A-V block	17
0.012	Monkey	Sustained physical performance, ED50	32
0.0125	Dog	Decreased running time on treadmill (RD1 = 0.006)	33
0.0125	Chimp	Behavioral changes	17
0.015	Dog	P- and T-wave changes	17
0.015	Rabbit	Mydriasis	34
0.017	Monkey	Conditioned avoidance response, ED50	32
0.020	Goat	Mydriasis, MED	34
0.025	Dog	Conditioned avoidance response	33
0.025	Dog	Mydriasis	34
0.037	Monkey	Visual discrimination	32
0.048	Dog	Prostration dose (PD1)	19
0.05	Cat	Mydriasis, MED	34
0.05	Dog	Hyperventilation, dry mouth, ataxia & confusion	17
0.1	Monkey	Increased heart rate, mydriasis & cycloplegia	17
0.1	Dog	Bradycardia	19
0.48	Dog	PD50	19
3.8	Monkey, rabbit	PD50	19
4.2	Swine	PD50	19
4.3	Swine	LD50	34
6.6	Goat	LD50	34
9.4-11.1	Dog, monkey, rabbit, cat, guinea pig	LD50	11, 17
18 - 25	Mouse	LD50	11

(Sim, Reference 19)

LD1s resulting from i.v. administration of BZ generally vary between 2 and 7 mg/kg. In most species, prostration occurs between 3 and 5 mg/kg. However, the PD50 (the dose that causes prostration in 50 percent of the exposed population) for the dog is 480 μg/kg and the PD1 is 48 μg/kg [19]. Minimal effective doses also differ substantially among the species. For example,

dogs may become ataxic at i.v. doses as low as 7 μg/kg while ataxia occurs at 3.2 and 2.0 mg/kg for rabbits and guinea pigs, respectively [17]. Of the animals tested, the dog is among the most sensitive to BZ; and according to Sim [19], its dose response is close to that of humans. Table 7 shows the progression of symptoms that occurred with increasing i.v. doses during a study of the effects of BZ on behavior [17]. Tachycardia occurred at a lower dose than other symptoms examined including electrocardiographic changes.

TABLE 7

EFFECT OF SINGLE I.V. INJECTIONS OF BZ IN THE DOG

DOSE (μg/kg)	EFFECT
5	Minor changes in heart rate
10	Marked increase in pulse rate (Occurred at rest and after jumping.)
10-15	Transitory electrocardiograph changes (A-V block)
12.5	Decreased running time on treadmill.
15	Electrocardiograph changes (P- and T-wave changes)
25	Interfered with ability to respond to learned signal (conditioned avoidance response).
50	Hyperventilation, hyperactivity, dry mouth, ataxia, and confusion

Data from McNamara [17]

Sim [19] noted that the same i.v. dose produced ataxia in dog and man, and the amounts that caused increased heart rates and interference with sustained physical performance were in the same relative order in both species. He used the similarity between low dose responses to i.v. BZ in man and dog as the basis for an estimation of the human LD50. Applying the ratio of 7:12 derived from estimated ID50s of 7 μg/kg for man and 12 μg/kg for dogs, to the LD50 of 9.6 mg/kg in the dog, he obtained an LD50 of 5.6 mg/kg for man. Using the same approach for prostration doses, he estimated the PD1 to be 0.028 and the PD50 to be 0.28 mg/kg in man.

Effects on Behavior

Certain aspects of behavior and learned responses in animals are quite sensitive to BZ. In general, behavioral effects occur in the same dose ranges that produce physiological effects. Single BZ doses which affected behavior in several species are shown in Table 8.

TABLE 8

THE EFFECTS OF BZ ON BEHAVIOR

Species	Dose (mg/kg)	Route	Effect	Reference
Chimp	0.0125	iv	Noticeable changes in behavior	17
Monkey	0.012	iv	Depressed sustained physical exercise (ED50)	32
	0.017		Depressed CAR (ED50)	
	0.037		Depressed visual discrimination test (ED50)	
Dog	0.0125	iv	Affected performance on treadmill	33
	0.025		Depressed CAR	
Dog	0.01	--	Lowest dose to affect normal behavior	11
Cat	0.005	sq	Depressed learned behavior	35
Rat	0.01	sq	Enhanced learned behavior (lever pressing in quest for water)	36
	0.05		Depressed learned behavior	
Rat	0.03	ip	Lowest dose to affect normal behavior	11
Rat	0.1	--	No effect on aggressive behavior	37
	4.5		Suppressed aggressive behavior	

-- = Route of administration not provided in source materials.
CAR = Conditioned avoidance response.

Liu et al. [36,38] showed that BZ elicits a biphasic effect on spontaneous motor activity and behavior in the rat. Low subcutaneous (s.q.) doses (0.01 mg/kg and 0.1 mg/kg for the rat and mouse, respectively) depressed spontaneous motor activity while higher doses (0.5 and 1.0 mg/kg for the rat and 0.3 to 10 mg/kg for the mouse) enhanced spontaneous motor activity in a dose dependent manner.

The BZ effects on spontaneous motor activity were paralleled by changes in learned behavior. BZ doses that depressed spontaneous motor activity also increased lever pressing in quest for water. Likewise, doses that increased spontaneous motor activity, decreased lever pressing.

Lowy et al. [35] found that BZ had a marked effect on learned behavior in cats trained to press a lever in response to an auditory signal. A single dose of 5 μg/kg caused this learned response to nearly disappear within 1 hour. Recovery was not complete until 5 to 7 days after the exposure. Pretreatment with atropine did not affect the behavioral effects of BZ.

Multiple Exposures

Multiple dose studies, which examined behavioral, physiological, and biochemical effects of BZ, have been conducted using different routes of administration in a number of animal species [17]. In some studies, tolerance to BZ was evident and, following multiple exposures, toxicologic symptoms were progressively slower to appear, less severe, and of shorter duration. Investigations of the effects of BZ on various parameters, including blood, kidney, and liver function, revealed no important changes at low doses.

Several studies demonstrated that, despite the irrefutable development of tolerance, repeated exposure to BZ did not alter the LD50. Mice were given up to 8 daily doses of 20 mg/kg i.p. which caused excitement in some and depression in others. No changes in body weight were noted throughout the 8-day period. At the end of the exposure period, mice were challenged with 60 mg/kg i.p. The mortality rate of mice previously exposed to BZ did not differ significantly from controls [17].

Similarly, the LD50 in six dogs treated with 100 μg/kg i.v. for 14 consecutive days was the same as in untreated controls. Tolerance was evidenced in these dogs by the increase in the time of onset of ataxia from an average of 4 minutes after exposure on the first day of dosing to 14 minutes after exposure by day 14. In another group of dogs treated with 100 μg/kg i.v., ataxia and hind leg weakness were no longer seen after 8 daily exposures [17].

A group of dogs was sacrificed and examined after 42 days of exposure to 100 μg/kg i.v. Slight pathological changes were observed in the gastrointestinal tract (ulceration, blood in the stool, and hyperemia of the muscle coat) in 50 percent of the test animals and 25 percent of controls. Treated dogs had a slight increase in kidney weight and a slight decrease in liver and spleen weight. There were no significant changes in packed blood volume, differential white blood cell count, or serum sodium concentration [17].

In another study, mice, dogs, and monkeys were exposed to BZ 5 days/week for 6 weeks. The oral, i.m., and i.v. doses administered are shown in Table 9. There were no changes in blood (hemoglobin, hematocrit, erythrocyte sedimentation rate, white blood cell and differential counts, blood glucose), liver function (bromosulphthalein), or kidney function (nonprotein nitrogen, BUN) [19].

TABLE 9
EFFECTS OF 6-WEEK EXPOSURES TO BZ

Route	Dose (mg/kg)	Remarks
	MOUSE	
Oral i.m. i.v.	12.5 - 200 3.1 - 50 0.63 - 10	Mice that survived for 6 weeks had normal body weights. No gross or histopathologic changes or deaths attributable to BZ.
	DOG	
Oral i.m. i.v.	1 - 50 0.1 - 5.0 0.1 - 2.5	Tolerance developed within 3-4 days. No changes in blood, liver, or kidney function. No gross or histopathologic changes or deaths attributable to BZ.
	MONKEY	
Oral i.m. i.v.	1 - 20 0.1 - 2.5 0.1 - 2.5	Only minimal signs of toxicity seen at high doses. No changes in blood, liver, or kidney function. No gross or histopathologic changes or deaths attributable to BZ.

Data from Sim [19]

Ataxia, which occurred least frequently with oral exposures, was the first sign to appear with all routes of administration in the dog. Other signs of central nervous system depression were decreased normal muscle activity and muscular incoordination. Dogs appeared to become tolerant to BZ within 3 to 4 days of exposure. The tolerance was partially lost during weekend breaks without drugs and then returned during the ensuing week of drug treatment. The level of tolerance did not change after the second study week.

Monkeys experienced only minimal drug effects. Dilation of the pupil, the only consistent symptom, was observed with all routes of administration and doses. These effects were least pronounced after oral administration of BZ. Neither ataxia nor disturbed behavior were observed, and the monkeys appeared to be healthy for the duration of the study period.

Tolerance to BZ was observed in two inhalation studies in dogs. In the first, two of four dogs survived 4-week exposures of 5 days/wk for 8 minutes each to 550 mg min/cu m. Ataxia declined after the fourth exposure and was not pronounced by the end of the study. Both surviving dogs had mydriasis. In the second study, five dogs were exposed up to 32 times over a 46-day period to daily Ct's ranging from 359 to 598 mg min/cu m. Mydriasis, ataxia

and prostration occurred in most dogs during the first five exposures. Only one dog showed signs thereafter; it died shortly after the twenty-third exposure. Survivors showed no significant changes in hematocrit, white-blood cell counts, differential counts, sedimentation rates, or liver function [17].

Mice repeatedly exposed to a range of BZ concentrations did not develop tolerance. These animals exhibited great activity and excitement which began within 1 or 2 minutes of exposure and lasted for 2 to 3 hours [17].

Repeated exposure may also lead to the development of a limited tolerance to the behavioral effects of BZ. Lowy et al. [35] found that BZ had a marked effect on learned behavior in cats trained to press a lever in response to an auditory signal. The performance level dropped almost completely after a single dose of 5 μg/kg and did not return to normal until 5 to 7 days later. A series of four single injections of 5 μg/kg at 10-day intervals caused a significant decrease in the duration of the behavioral effects with each subsequent trial. In one of five cats tested, the duration decreased from 9 days after the first dose to 3 days after the fourth dose. Tolerance was limited to the duration of the response since repeated doses of BZ caused no apparent diminution in the severity of the behavioral effects occurring within the first few hours after exposure.

A tolerance to the effects of BZ on the CAR was observed in one dog treated 5 days/week for 2 weeks with 50 μg/kg i.v. The effect on CAR decreased from a duration of 4 hours on the first day of dosing to 2 hours on the fifth day where it remained until the end of the 2-week treatment period [17].

In summary, repeated administration of BZ may induce tolerance to its physiological and behavioral effects in dogs and to behavioral effects in cats. No tolerance was seen in multiple dose studies with mice and monkeys, and repeated doses did not alter the LD50 in any species.

CONCLUSION

Studies of the effects of BZ in humans focused on single exposures in which BZ was administered by ingestion, inhalation, or injection (i.v. or i.m.). These studies showed that BZ can cause mild incapacitation with single i.m. or i.v. doses as low as 2 μg/kg [9]. Two multiple dose studies have been conducted in humans [8, 22]; only one of these [8] is applicable to the estimation of an MPC for field drinking water. In this study, three consecutive daily BZ doses of 1 μg/kg caused no significant effects. However, by the fourth day, signs of BZ intoxication became apparent and subjects were mildly incapacitated by the fifth day. No symptoms suggestive of BZ intoxication were apparent in men given 0.5 μg/kg for 6 days of the 8-day study period. This data served as the basis for a water potability standard developed in 1963 in which 0.5 μg/kg was considered to be a safe dose for consecutive daily exposures [25]. No new studies have been performed since that time which would indicate a change in the maximum safe dose of 0.5 μg/kg. A risk assessment diagram based on human studies is presented in Figure 3.

Toxicity studies were performed in a number of animal species. Although somewhat more resistant to the effects of BZ than man, the dog is among the most sensitive of the animal species tested; and, according to Sim (19), the response of the dog is most like that of humans. However, unlike man, in which successive BZ exposures had a cumulative effect, the dog clearly developed tolerance to consecutive BZ exposures [17]. This fact alone negates the potential for extrapolating animal exposure data to the development of standards for human water consumption.

Some of the available animal data indicate that repeated exposures to low doses of BZ for short time periods should not produce organ damage or histopathologic lesions that are threatening to health. In monkeys and dogs exposed for periods of 6 weeks to daily i.v doses as high as 2.5 mg/kg, and daily oral doses as high as 20 and 50 mg/kg, respectively, no changes in blood composition, or liver and kidney function were noted (19). No other gross or histopathologic lesions were observed that were attributable to BZ.

In another study, slight pathological changes were observed in the gastrointestinal tract of a group of dogs after 42 days of exposure to 100 μg/kg i.v. These lesions were observed in 50 percent of test animals and 25 percent of controls. The appearance of the lesions in the control dogs suggests that the stress of the chemical treatment may have enhanced an intercurrent infection in the BZ-treated animals. While there were no changes in the composition of the blood, treated dogs also had slight changes in organ weights [17].

According to Sim's analysis [19], the incapacitating dose for the dog is less than twice that for humans. Therefore, the dose which caused slight changes in pathology and organ weight in the second study cited above is equivalent to at least 100 times the dose of 0.5 μg/kg in humans. This should be a sufficient safety margin to assure that pathologic lesions would not develop in humans exposed to 0.5 μg/kg for a period of 7 days. The lack of any pathology in the first dog study cited above supports this premise.

However, the pathological changes noted during the second 42-day dog study, indicates that organ damage may be possible with this compound. Therefore, it is necessary to assure that lower doses would not cause such changes if administered for an extended period of time before permitting human exposures greater than 1 week. Since 6 weeks is the longest time over which BZ was administered repeatedly to animals, there is insufficient data to judge the possible effects of low levels of BZ in drinking water for an extended period. Therefore, due to lack of sufficient data in animals or man, a long-term drinking water standard (>7 days to <1 year) cannot be recommended at this time.

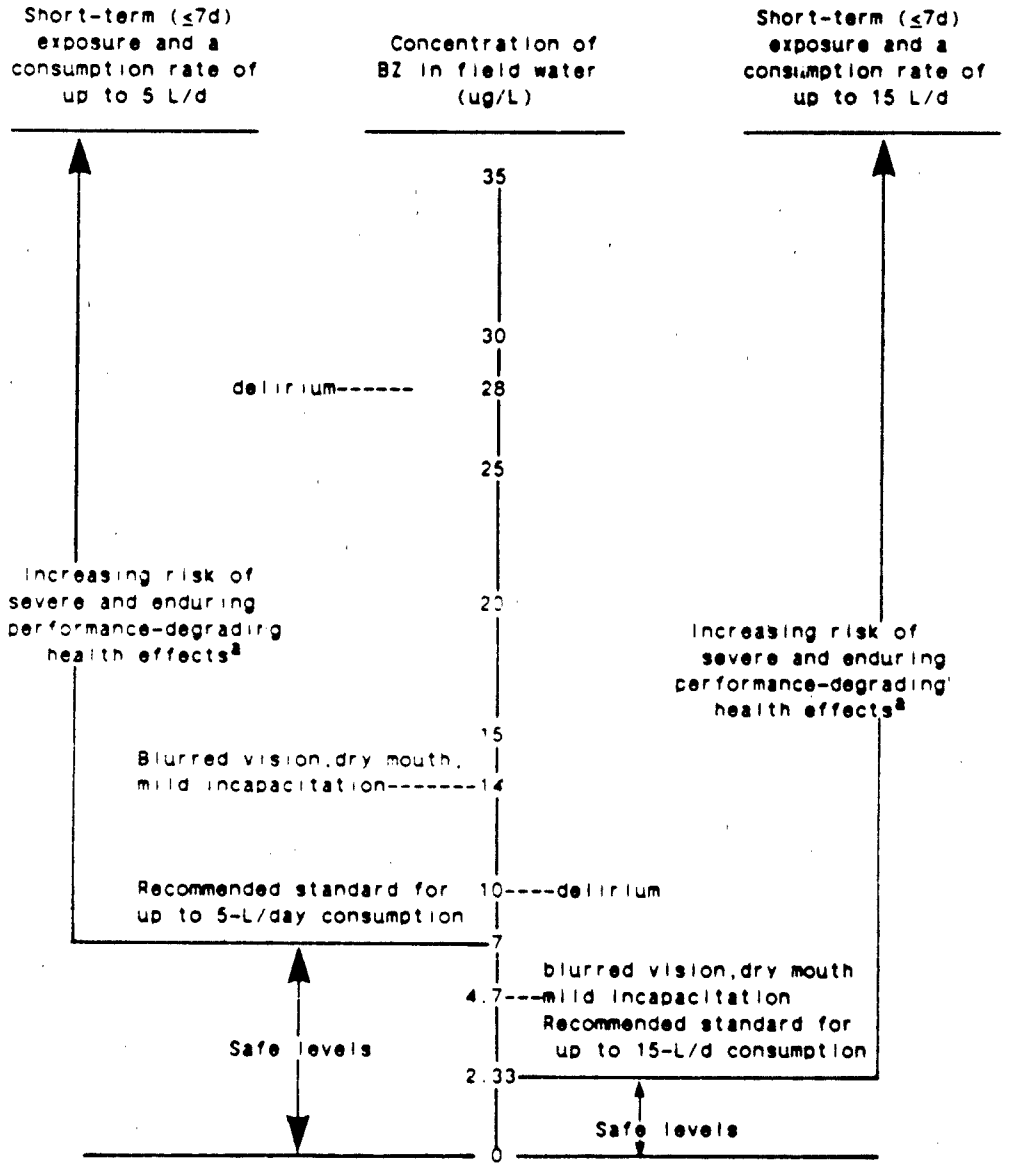
The 7-day standard is calculated assuming a weight of 70 kg and a maximum safe dose of 0.5 μg/kg. The short-term drinking water standard (<7 day exposure) for a daily consumption of 5 liter water is 7 μg/l.

$$\frac{(70\ \text{kg})\ (0.5\ \mu\text{g/kg})}{(5\ \text{liter})} = 7\ \mu\text{g/l}$$

The 7-day drinking water standard for a daily consumption of 15 liter water is 2.33 μg/l.

$$\frac{(70\ \text{kg})\ (0.5\ \mu\text{g/kg})}{(15\ \text{liter})} = 2.33\ \mu\text{g/l}$$

The only safety factor built into these standards is the 10 [26] to 25 percent [25] estimated difference in the effectiveness of oral and i.v. (or i.m.) doses. The short-term drinking water standard for a consumption rate of 5 liter/day is the same as that proposed in 1963 [25]. Since heat can increase the severity of the effects of BZ [21], an association between elevated temperatures and an increase in performance decrement with progressive exposures over a 7-day period can be anticipated. This may be especially important for persons wearing-chemical protective clothing.



[a]Performance-degrading health effects may include rapid pulse, decreased salivation, blurred near vision, decreased mental performance, poor coordination, restlessness, stupor, hallucinations, delirium.

Figure 3. Health-effect summary for BZ in field water.

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1 U.S. Air Force Engineering Services Center
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1 Naval Sea Systems Command
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