BRUCE STANHOPE, NEAL NETZEL

Velsicol Chemical Corporation
Northbrook, IL 60062-2005
U S A

An environmental, health and safety overview of benzoate plasticizers*)

Summary — A review concerns the characteristic of the novel plasticizer Benzoflex® 2088 composed of three already known benzoate plasticizers: diethyleneglycol dibenzoate (Benzoflex® 2-45), dipropyleneglycol dibenzoate (Benzoflex® 9-88) and triethyleneglycol dibenzoate (Benzoflex® S-358). Detail analyses of these components show that Benzoflex® 2088 creates no health hazard (*e.g.* lethal dose LD_{50} is 3—5 g/kg of body weight). Data concerning its estrogenic activity, metabolism, toxicity to aquatic organisms and biodegradability were presented. Plasticizer investigated quickly undergoes biodegradation in the environment and does not show the tendency to accumulate in the organisms.

Key words: benzoate plasticizer "Benzoflex® 2088, health and safety, effect on the environment.

Benzoate plasticizers have been used in a number of industrial applications, such as plasticising PVC, for many years. As with many products introduced years ago, the environmental and toxicological testing protocols used at the time of their introduction, are not consistent with today's standards. Over the last five years, a significant amount of this testing has been performed on several benzoate plasticizers. Three of these plasticizers, when blended, form a preparation commonly known as Benzoflex® 2088.

All recent testing was conducted according to OECD and USEPA protocols and standards, where the methods have been standardized. Each benzoate was tested independently. As expected, each component produced similar results. In this paper, the following attributes will be discussed:

— composition and physical-chemical properties of Benzoflex® 2088,

— review of potential health effects including recent reproductive and developmental data,

— low toxicity and rapid degradation in the aquatic environment.

COMPREHENSIVE CHARACTERISTIC OF BENZOFLEX® 2088

Benzoflex® 2088 is composed of three benzoate plasticizers. These plasticizers include diethyleneglycol dibenzoate (Benzoflex® 2-45), dipropyleneglycol dibenzoate (Benzoflex® 9-88) and triethyleneglycol dibenzoate (Benzoflex® S-358). Table 1 gives the physico-chemical properties of each of the components.

T a b l e 1. Physico-chemical properties of Benzoflex® 2088 components*)

	Benzoflex® 2-45	Benzoflex® 9-88	Benzoflex® S-358
Water solubility, mg/L	38.3	8.69	30.4
Henry's law, atm · m^3/mol	$7.0 \cdot 10^{-10}$	$3.8 \cdot 10^{-8}$	$1.8 \cdot 10^{-9}$
$Log_{10}P_{ow}$**) (HPLC method)	3.2	3.9	3.2
$Log_{10}K_{oc}$**)	3.2	3.6	3.2

*) Not: corrosive, oxidizing, explosive or reactive (Directive 67/548/EEC).
**) The meanings of symbols — see text.

As the data illustrates, these benzoates have low water solubility and are not volatile materials. They also have relatively low log_{10} octanol/water partition coefficients (P_{OW}). Their low water solubility makes testing these substances in water particularly difficult because they are also ready biodegradable, which will be presented later in this paper.

The partition coefficient (K_{OC}) is a measure of affinity of a substance to absorb onto soil. A low K_{OC} (water to organic carbon) indicates a relatively low soil preference, whereas a high K_{OC} indicates that the test substance is not mobile in soil. As the data indicates, the benzoates are likely to adhere to soil but are not permanently bound.

*) Lecture presented during International Conference "PVC 2002 — Toward a Sustainable Future", 23—25 April 2002, Brighton, Great Britain.

TOXICOLOGY OF BENZOFLEX® 2088

When evaluating the toxicology of a product, there are generally four categories of studies that are considered: acute, sub-chronic (sometimes referred to as repeated dose), multi-exposure multi-generation and specialty studies. Included in the multi-exposure studies would be evaluations related to the reproductive system, developmental toxicology and effects on fertility. Specialty studies often include an evaluation of estrogenic activity, mutagenicity and other genotoxicity studies, and metabolism.

Acute studies

The acute studies for each dibenzoate are summarized below:

— low acute toxicity (rat, oral, LD_{50},) — 3914 to 5313 mg/kg (combined males/females),
— low acute dermal toxicity (rat) — >2000 mg/kg,
— not a skin irritant (rabbit),
— not an eye irritant (rabbit),
— not a skin sensitizer (guinea pig).

The acute toxicity of a substance reflects its ability to cause effects after a single, usually large, dose. The results of acute testing are generally regarded as predictors of effects that might be observed in an emergency situation or where the potential for a single, large exposure might occur. The testing of the components of Benzoflex® 2088 indicates that the material is relatively nontoxic from an acute standpoint and therefore unlikely to cause acute effects even when a relatively large, single dose exposure might occur. The product is not considered acutely toxic within the OSHA Hazard Communication Standard definition nor under the European Directive 67/548/EEC either by oral, dermal, inhalation, eye irritation/corrosion, skin irritation/corrosion or skin sensitization.

Repeated dose studies

To evaluate the repeated does effects of a substance, 90 day feeding studies are generally conducted and the results of these studies are expressed in terms of a "No Observed Effect Level" (NOEL), that is, the level at which exposure takes place repeatedly for 90 days without causing an observed effect. These studies typically include an examination of multiple organs and systems including the liver, spleen, and kidney, and seeking evidence indicative of a dose-related response. Each of the components of Benzoflex® 2088 were evaluated in 90 day studies [oral feeding as doses up to 2,500 mg/(kg · day)]. The results are given below:

— NOEL 1000 mg/(kg · day) (all components of 2088),
— NOAEL >2500 mg/(kg · day) (2-45 and 9-88) (NOAEL = No Observable Adverse Effect Level),
— NOAEL >2200 mg/(kg · day) (S-358),
— all treatment related affects were reversible or trending to reverse,
— primary treatment related effects was low body weight gain,
— reversal (or trending to reverse) occurred during 4 week recovery group.

So, NOEL was 1000 mg/(kg · day). Just as importantly, findings at higher doses, up to and including 2500 mg/(kg · day), were shown to be reversible after a 4 week recovery period. For a frame of reference, a dose of 1000 mg/(kg · day) for a 70 kilogram person would be a dose of 70 grams. These repeated dose studies reinforce the products low hazard potential.

Special studies

A number of what might be considered special studies have been conducted on the components of Benzoflex® 2088 including studies to evaluate the estrogenic activity, mutagenicity and metabolism.

Endocrine disruption

Each of the components of Benzoflex® 2088 were tested for estrogenic mimicking or endocrine disruption effects. The testing conducted on the substances of the preparation used generally accepted endpoints for assessing estrogenic activity *in vivo* and were shown to have no endocrine disruption nor any estrogen mimicking effects.

Mutagenicity

Mutagenicity studies, conducted on compounds to determine their ability to cause effects at the cellular level, which can lead to genetic disease or cancer, have also been conducted on the components of Benzoflex® 2088. In the standard Ames test, none of the substance exhibited evidence of toxicity nor mutagenic activity. In another set of studies called "Mammalian Cell Mutation" studies, the components all tested negative at inducing mutation effects.

Metabolism

Metabolism studies are conducted to determine what happens to a compound after it is ingested, how it is changed or converted by the body and how it is eliminated from the body. The determination of a substance ability to bioaccumulate is determined through metabolism studies. These studies also provide insight into the metabolites themselves. A "safe" compound can be converted into potentially more toxic metabolites as it passes through living systems. A metabolism study which examined the adsorption, desorption, metabolism and excretion has been conducted on diethyleneglycol dibenzoate, with very favorable results. The diethyleneglycol dibenzoate is quickly absorbed following oral administration and then rapidly metabolized and excreted

with urinary excretion of the metabolites occurring in less than 24 hours. Of significance is this metabolism study not only indicates that the bio-accumulation of this compound is unlikely but also that its metabolites do not accumulate either. Thus, adding to the safety of this product, there is no evidence that diethyleneglycol dibenzoate will accumulate in tissue and no evidence that the substance or its metabolites are persistent in the body. Based on the similarity of this substance with the other glycol dibenzoates, these results indicate that the chemically related dibenzoates cal also be expected to exhibit only extremely low potentials of accumulating in the body.

During all of the testing conducted on the substances comprising Benzoflex® 2088, no unusual infections or diseases in any of the animals were observed. The subchronic studies, which examine all immune system organs and blood parameters including a measure of immune proteins, show no indication of immune system compromise. Therefore, while the components of Benzoflex® 2088 have not been specifically tested for immune system response, it is highly unlikely that such immunosuppressive activity is an inherent property of these compounds.

Chronic and reproductive studies

A significant number of studies have been conducted on the components of Benzoflex® 2088 to assess their effects on reproduction, including developmental toxicity studies and multi-generation reproduction studies. Developmental studies are designed to detect a substance's ability to cause birth defects on the offspring of individuals (in this case, mice) exposed to a compound during pregnancy. The results of these studies were as follows:

— tested up to 1000 mg/(kg · day) during gestation,
— maternal NOEL 1000 mg/(kg · day),
— developmental NOEL 500 mg/(kg · day) (Benzoflex® 2-45/Benzoflex® S-358),
— developmental NOEL 250 mg/(kg · day) (Benzoflex® 9-88).

The effects on pre-natal development were primarily limited to incomplete ossification, *i.e.*, slight retardation of growth, which is not considered adverse. Based on this data, these substances are not considered developmental hazards.

Multi-generation reproductive studies evaluate the effects of the test substances on parameters such as mating performance, fertility, estrous cycling, sperm mobility and other processes. The results in Table 2, summarize the investigations of Benzoflex® 9-88 and Benzoflex® 2-45.

Table 2 illustrates that neither of the substances adversely affected reproductive parameters at dietary levels up to 10 000 ppm. This corresponds to a level of 800—1500 mg/(kg · day), depending on weight and food consumption of the animals at different stages of the study.

Table 2. Benzoflex® plasticizers 2-generation reproductive toxicity

	Diethylene glycol dibenzoate	Dipropylene glycol dibenzoate
Parents and reproductive parameters*)	10 000 ppm NOAEL	10 000 ppm NOEL
Offspring**)	3300 ppm NOAEL	10 000 ppm NOEL

*) NOAEL = lower bodyweight gain days 0—4 after litters born; comparable at end of lactation (parents).
**) Lower body weight day 1 and 14—21 (offspring).

TOXICITY TO AQUATIC ORGANISMS

Groups off fish were exposed to water accommodated fractions (WAFs) under semi-static conditions with 24-hour renewal at 20°C. The results of these tests are given in Table 3. These data represent the lethal loading that caused 50% mortality in fish and 50% effect in *Daphnia magna* and *Selenastrun capricornutum*. The formulation methods followed protocols for the testing of difficult substances.

Table 3. Benzoflex® plasticizers ecotoxicity

Test system	Benzoate 2-45	Benzoate 9-88	Benzoate S-358
Fathead minnow, 96 h, LC_{50}	3.9	3.7 (FT)*)	>100
Rainbow trout, 96 h, LC_{50}	2.9	>3.0 (FT)*)	16
Daphnia magna, 48 h, EC_{50}	6.7	19.3	26
Selenastrum capricornutum, 72 h C_{50} (biomass)	5.2	1.1	33

*) FT = flow-through conditions.

Exposure levels in aquatic tests are extremely difficult to achieve and maintain even under acute (no feeding) conditions due to rapid biodegradation; therefore alternative methods were employed such as flow-through techniques. However, even with high volume replacement and renewal of tanks each day, microbial numbers increase substantially during aquatic tests, presumably because of bacterial re-inoculation by fish.

READY BIODEGRADABILITY

Ready ultimate biodegradability was assessed using the CO_2 evolution test. Substances are considered to be readily biodegradable if CO_2 production is equal to or greater than 60% of the theoretical value within 10 days of the level achieving 10%. Each of the three benzoates achieved the required pass level so they were classified as readily biodegradable. The final levels of biodegradation achieved by the end of the test were sufficiently

high to indicate that the substances were completely mineralised.

The ultimate anaerobic biodegradability of each substance was assessed by the measurement of headspace biogas production. Substances are considered to be ultimately biodegradable in this test if the level of biodegradation achieves 60% of the theoretical level by the end of the test. All three benzoates achieved the required pass level and were classified as ultimately biodegradable under anaerobic conditions.

Additional degradation studies were conducted to assess the rate and extent of biodegradation in natural water. These studies were designed to determine the rate and extent to which each substance degraded. This was accomplished by measuring the concentrations of the principal components and degradates in mixtures incubated at temperatures in the range of 20 to 24°C. The results of these studies were similar for all three benzoates tested. In each case the parent compound was readily degraded (half-lifes were 6 to 28 hrs) and none of the degradates were persistent. Since neither the parent or degradates are persistent in natural water, they are unlikely to present any potential for long-term danger to aquatic organisms.

LOW BIOCONCENTRATION POTENTIAL

A standard bioconcentration factor (BCF) study is not possible with these substances as they degrade too rapidly to maintain a steady exposure concentration, which is a pre-requisite for a valid test (OECD guideline 305, 14 June 1996). Therefore a modelling program was utilized to predict the BCF for these benzoate substances. After validating the model, BCF factors were determined for each substance. The BCF results ranged from 27 to 193 for the three substances. The BCF for the degradates were predicted to be less than 1. As a reference, substances with a BCF factor of less than 100 are not considered bioaccumulative.

CONCLUSIONS

Though Benzoflex® 2088 is a relatively new product, its components are not. The three components of Benzoflex® 2088 are structurally related dibenzoate esters. The testing performed on the Benzoflex® 2088 components is comprehensive, recent, and conducted according to EU, OECD and USEPA protocols. As discussed these substances showed the following results:

— Low acute toxicity with the oral LD_{50} between 3—5 g/(kg · day).

— NOEL for subchronic toxicity is at least 1000 mg/(kg · day).

— Reproductive toxicity (2-generation) showed no evidence of adverse effects at 10 000 ppm (two of three components were evaluated).

— No estrogenie activity.

— ADME (absorption, desorption, metabolism, excretion) of major component shows no persistence in the body (rat).

— Prenatal development indicated the most conservative NOEL of 250 mg/(kg · day) for one of the components (other components were higher); the effects were not considered adverse.

Based on the toxicology data, some of which is presented above, Benzoflex® 2088 is extremely unlikely to pose an adverse health risk. This interpretation is supported by a recent risk assessment.

Benzoflex® 2088 is also readily biodegradable and does not form persistent residues under either aerobic or anaerobic conditions. Studies of acute aquatic toxicity also highlighted that these substances are extremely biodegradable causing the use of extraordinary test methods to maintain a concentration long enough to complete the testing.

The results of acute aquatic toxicity tests give 50% effect concentrations for lethality, immobility or biomass reduction that are typically in the 1—10 mg/L range for two of the components and 10—100 mg/L range for the third. Only one component has acute toxicity values that are within its limit of aqueous solubility.

The above results clearly illustrate the low toxicity of the components of Benzoflex® 2088, their inability to accumulate in the body, and their rapid biodegradation in the environment.