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# Vapor-Phase Toxicity of Butylbenzyl Phthalate to Three Plant Species: White Mustard, Chinese Cabbage, and White Clover

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**Abstract** During the manufacture of products containing butylbenzyl phthalate (BBP), low emissions to the air may occur. Due to potential exposure of terrestrial communities to BBP vapors, phytotoxicity tests were conducted using Chinese cabbage, white mustard, and white clover. No significant effects on shoot growth were observed at the higher BBP vapor-phase concentration tested, which measured 5.7 μg/m$^3$. The overall practicality of vapor-phase testing of chemicals with very low vapor pressures is reviewed. These study results suggest that environmental risk from exposure to BBP vapor is negligible for plants.

**Keywords** Vapor-phase phytotoxicity · *Sinapis alba* · *Brassica campestris* · *Trifolium repens*

Phthalate esters have been commercially available for more than five decades, and are widely used in plastic products. Very little phthalate ester is lost to the environment, including the air compartment, and the majority of those phthalates in the environment are due to the slow release from plastics (Stanley et al. 2003). Although reviews on the effects of phthalate esters to aquatic organisms are data rich (Adams et al. 1995; Staples et al. 1997), far fewer studies have been performed with plants. Plants, being primary producers, are an essential component of a healthy environment, and determining the effects of contaminants on them is a crucial step in assessing the potential risk to the terrestrial ecosystem (Gorsuch et al. 1991). Phthalate ester phytotoxicity data are reviewed by Parkerton and Staples (2003) and by Gorsuch et al. (2001). Butylbenzyl phthalate (BBP) is a plasticizer commonly used for vinyl flooring, sealants, paints, and trace amounts have been measured in air adjacent to manufacturing sites. Regional outdoor air BBP concentrations have been confirmed by analysis with mean air concentrations in Europe, United States, and Canada of 1.7, 9.5, and 1.78 ng/m$^3$, respectively (Clark et al. 2003). In their publication, Cole et al. (1984) reported adverse effects on plants (e.g., chlorosis, reduced growth, and death) from the vapors of the structurally similar dibutyl phthalate (DBP).

Cabbage (*Brassica* sp.) and white clover (*Trifolium repens*) seedlings were found to be particularly sensitive to DBP vapors at very low (0.2–0.9 μg/m$^3$) concentrations (Hardwick et al. 1984; Dueck et al. 2003). As a result of these findings, phytotoxicity studies exposing *Brassica* sp. and *T. repens* to BBP vapors were conducted at target concentrations of 1 and 10 μg/m$^3$.

## Materials and Methods

No standardized methods exist for conducting vapor-phase plant studies. The study was based on OECD Test Guidelines 208 (OECD 1984) and 227 (OECD 2006), modified for gaseous compounds. The study followed good laboratory practices (OECD 1998). Based on their reported

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sensitivity to the structurally similar DBP vapors, three dicotyledonous plant species were evaluated: white mustard (*Sinapis alba*, formerly *Brassica alba*), Chinese cabbage (*B. campestris var. chinensis*), and white clover (*T. repens)*. Seeds were germinated in a sandy loam soil (standard soil SP 2.3, Speyer, Germany, 0.7% organic matter and pH 6.5 ± 0.2). After 7–12 days, plants uniform in size with at least one mature leaf were transplanted in 200-mL cardboard pots containing the sandy loam. One mustard plant or one Chinese cabbage plant was added per pot (replicate), while 20 white clover plants were added per pot. Eight pots of each plant species (24 pots per chamber) were placed in a tray used for subirrigation. Water was added as needed prior to exposure, while nutrient solution was pumped into the tray daily during the 21-d tests. The nutrient solution contained 200 mg/L $Ca(NO_3)_2 \cdot 4H_2O$, 50 mg/L each of $KNO_3$, $KH_2PO_4$, and $MgSO_4 \cdot 6H_2O$, 25 mg/L KCl, and ca. 10 mg/L $FeCl_3 \cdot 6H_2O$. Excess solution was drawn off.

The plant exposures were conducted at AQura GmbH, Marl, Germany using four 230 L environmental chambers. Two control and two treatment chambers were established for each test and vapor-phase BBP was delivered to the two chambers for 21 days. Environmental chambers were equipped with SON-T ARGO 400-high pressure sodium-vapor lamps (Phillips) that operated on 16 h light (80–100 W/m$^2$), 1-h transition dawn and dusk (2 W/m$^2$), and 6 h dark. The temperature and humidity were controlled for daytime conditions (25 ± 2°C and 60–90%) and nighttime conditions (20 ± 2°C and 60–90%), respectively. Test plants were arranged randomly inside the environmental chambers.

After an exposure period of 21 days, the test was terminated and aboveground foliage (shoots) was harvested. Shoot fresh weights were immediately determined (mustard and Chinese cabbage individually; white clover 20 plants/pot) and recorded. Shoots were then dried (95°C for 40 h) and weights determined and recorded. Shoot mean fresh and dry weight values, and their standard deviations, were calculated per exposure treatment (control and BBP vapors) for each of the three plant species. Visual observations were made throughout the exposure period and recorded if abnormalities were noted.

A commercial grade sample of BBP (CAS no.: 85-68-7) with a purity of 98.5% was used. A constant stream of activated charcoal/porcelain filtered air was passed through the control chambers. Compressed air from gas cylinders was used for the BBP exposure chambers. In Experiment 1 the clear liquid BBP stock solution was placed in a vessel and cooled to 10°C in a water bath while in Experiment 2 the BBP stock was warmed in a water bath to 27°C before the compressed air was passed over it (see Fig. 1), carrying the BBP vapors to the mixing tank. To decrease the possibility of BBP aerosol transport, the BBP–air mixture passed through a third vessel filled with cotton wool/glass beads. To produce target concentrations, BBP was mixed with ambient temperature filtered air stream to achieve vapor exposure concentrations of 1 μg BBP/m$^3$ in Experiment 1 and 10 μg BBP/m$^3$ in Experiment 2. Fans within chambers provided sufficient turbulence to keep the BBP vapors circulated. BBP vapors were introduced into treatment chambers #2 and #3 while filtered air was pumped into the two control chambers at a flow rate of 2,000 L/h.

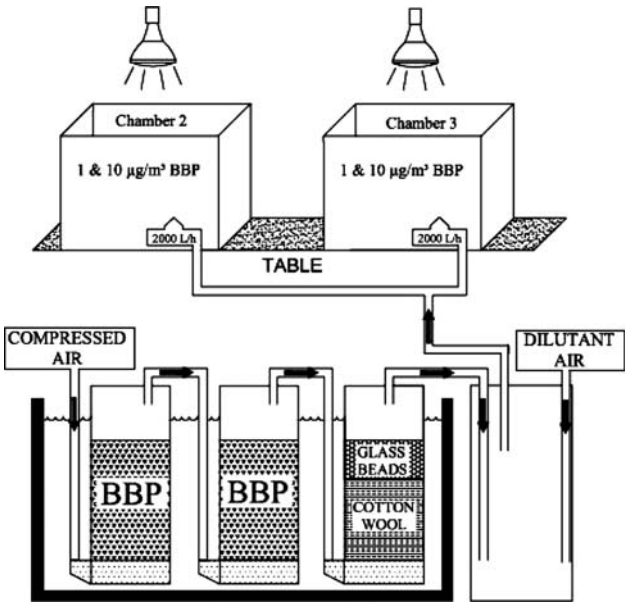


**Fig. 1** Airflow rate and temperature schemes to develop BBP vapor concentrations in exposure chambers. Experiment 1–100 L/h compressed air was bubbled through BBP cooled to 10°C in a water bath and mixed with 3,900 L/h of filtered, dilutant air to produce the nominal 1 μg/m$^3$ BBP vapor stream. Experiment 2–60 L/h compressed air was bubbled through BBP warmed to 27°C in a water bath and mixed with 3,940 L/h of filtered, dilutant air to produce the nominal 10 μg/m$^3$ BBP vapor stream

In addition to analyzing for BBP, concentrations of DBP, which had been found during some preliminary studies and is a known phytotoxic material, were determined once per week in the air of all four chambers. Control air and BBP vapor samples were collected at the plant canopy heights from the middle of each chamber and analyzed immediately. The air from control chambers was sampled once a week, while the air from chambers receiving BBP vapors was sampled five times per week. The analytical method described in Dueck et al. (2003) was suitable for the determination of BBP and DBP in air. A measured volume of sample air was drawn through a glass tube filled with Tenax® porous polymer adsorbent at a rate of 1 L/min to capture the BBP present in the air stream. All tubes were blank-tested for contamination before use. BBP was desorbed from Tenax® and analyzed by thermal desorption gas chromatography–mass spectrometry coupling. Internal and

external standards were used for each analysis series. The BBP detection and determination limits were 3 and 9 ng, respectively, based on 10 determinations. The determination limit for DBP was 5 ng. The limit of quantification for BBP and DBP in air was 0.2 μg/m$^3$. Quantitative determination of BBP and DBP were done by calibration against an internal standard (deuterized di-(2-ethylhexyl) phthalate).

Fresh and dry weights of control and BBP exposed shoots were statistically analyzed using the Dixon-Q outlier test (Sachs 1992), and outliers were excluded from further analysis. The fresh and dry weights were then evaluated for normal distribution and statistical differences ($\alpha = 0.01$) with the test of Wilcoxon, Mann, and Whitney (Sachs 1992). Growth was expressed as percent of controls.

## Results and Discussion

Physical conditions in the chambers remained within the target ranges. Measured BBP concentrations during the two 21-d plant tests are shown in Fig. 2. BBP concentrations were stable over the 21-d exposure period, averaging 0.47 ± 0.098 μg/m$^3$ in Experiment 1 and 5.7 ± 0.933 μg/m$^3$ in Experiment 2. BBP in the air stream was less than target concentrations possibly due to absorption on the cotton wool in the trap.

No adverse effects were observed on the shoot fresh or dry weights for any of the three species exposed to BBP vapors in either experiment compared to their respective control plants. In Experiment 2, the mean fresh and dry weights of Chinese cabbage and the mean fresh weights of white clover were significantly different ($\alpha = 0.01$) than the biomass of their respective control plants (positive effect). No differences were observed on the physiology or morphology of any exposed plant species compared to the control plants. Based on these two 21-d studies starting with 7- to 12-d post emergent seedlings, the BBP vapor-phase measured mean concentrations and standard deviations of 0.47 ± 0.098 and 5.7 ± 0.933 μg/m$^3$ did not cause any adverse effects on growth (biomass of foliage) or produce any observable adverse effects on coloration (e.g., chlorosis) or morphology of the three plant species. The mean fresh and dry weights of shoots are summarized in Table 1.

In the test with DBP lasting 62 days (Dueck et al. 2003), the Chinese cabbage shoot NOEC value was 0.90 μg DBP/m$^3$ and the white clover shoot NOEC value was 0.60 μg/m$^3$, suggesting a potential for effects with BBP; however, no adverse effects were observed in either species in this study at a concentration of 5.7 μg BBP/m$^3$. This illustrates that structurally similar chemicals do not always cause similar toxic responses at comparable concentrations.

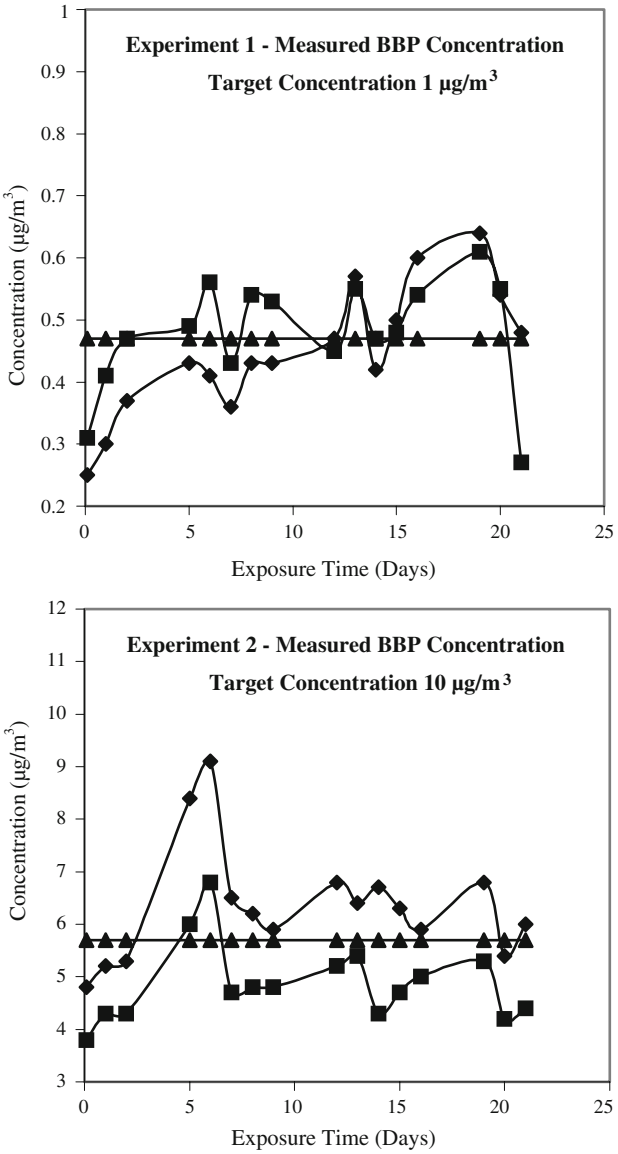


**Fig. 2** Measured BBP concentrations (μg/m$^3$) in the exposure chambers over the 21-d test period. (▲), Mean BBP concentration over 21-d exposure period (0.47 μg/m$^3$ in Experiment 1; 5.7 μg/m$^3$ in Experiment 2); (■), BBP concentration chamber 2; (◆), BBP concentration chamber 3

Potential adverse ecological effects from phthalate esters in the gaseous form (vapor) are strikingly different than phthalate esters in soil, sediment, or water (Parkerton and Staples 2003). Phthalate esters incorporated in soil present a much lower concern to plants. Soil microbes metabolize phthalate esters at moderately rapid rates (Peterson and Staples 2003). Therefore, absent repeated or continuous introduction of phthalate esters into soils, levels of phthalate esters decrease significantly over a period of several days to a few weeks. Lokke and Rasmussen (1983) concluded that even airborne phthalate esters were unlikely lead to significant accumulation in plants. The atmospheric

**Table 1** Mean and standard deviation (SD) of fresh weights (fwt) and dry weights (dwt) of the shoots and growth expressed as percent (%) of control plants, for Experiment 1 (0.47 μg BBP/$m^3$) and Experiment 2 (5.7 μg BBP/$m^3$)

| Endpoints | White mustard (*Sinapis alba*) | | Chinese cabbage (*Brassica chinensis*) | | White clover (*Trifolium repens*) | |
|---|---|---|---|---|---|---|
| | 0.47 μg/$m^3$ | 5.7 μg/$m^3$ | 0.47 μg/$m^3$ | 5.7 μg/$m^3$ | 0.47 μg/$m^3$ | 5.7 μg/$m^3$ |
| Mean fwt (g) of 16 sets control plants | 8.37 (0.99) | 6.63 (1.35) | 9.98 (4.16) | 10.36 (0.77) | 4.74 (1.87) | 6.67 (0.67) |
| Mean fwt (g) of 16 sets exposed plants | 8.80[a] 9.25[b] (1.24) | 6.66 (1.49) | 10.92 (2.35) | 11.02 (0.56) | 4.86 (1.59) | 7.25 (0.29) |
| Fresh weight growth exposed plants (% controls) | 105[a] 111[b] | 100 | 109 | 106[c] | 103 | 109[c] |
| Mean dwt (g) of 16 sets control plants | 1.29 (0.33) | 1.04 (0.28) | 1.10 (0.57) | 1.21 (0.15) | 0.55 (0.27) | 0.77 (0.12) |
| Mean dwt (g) of 16 sets exposed plants | 1.29 (0.39) | 1.04 (0.34) | 1.14 (0.38) | 1.39 (0.14) | 0.53 (0.20) | 0.84 (0.08) |
| Dry weight growth exposed plants (% controls) | 100 | 100 | 104 | 115[c] | 96 | 109 |

[a] Includes outlier in calculation

[b] One outlier (2.02 g) removed from calculation

[c] Statistically different ($\alpha = 0.01$) than the control plants (positive effect)

half-life of BBP is estimated to be 18 h (Peterson and Staples 2003) and therefore, emitted BBP would be rapidly reduced in concentration.

The results of this study with BBP and the recent study with DBP (Dueck et al. 2003) demonstrate that it is possible to conduct vapor-phase toxicity tests with sensitive plant species using compounds with very low vapor pressures. It was possible to bring the liquid BBP into a relatively stable vapor-phase throughout the two 21-d experiments. Published exposure studies using chemical vapor are quite limited due to their high cost to conduct, lack of standard test guidelines, and difficulty in maintaining stable chemical exposure concentrations throughout the test period (Kapustka et al. 2003). Collecting toxicity data on chemicals for risk assessment generally requires following standardized procedures. The present BBP experiments, using the OECD 208 and 227 test guidelines required substantial modifications to accommodate a vapor-phase exposure. Lack of standardization and the challenges associated with vapor-phase testing were summarized by Kapustka et al. (2003) who concluded that plants exposed to vapor-phase chemicals are generally more sensitive in the laboratory than in field studies. They also emphasized that the exposures should be environmentally relevant, stating that most often effects are only observed at unrealistically high concentrations.

The objective of this study was to determine whether BBP vapors would have a phytotoxic effect at target concentrations of 1 and 10 μg/$m^3$ on two sensitive plant genera (*Brassica* and *Trifolium*). In this study, no adverse effects were observed on plant seedlings exposed to BBP vapors at 5.7 μg/$m^3$; hence, based on the results from this study, adverse risk to plant communities is not expected from BBP vapors.

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