Residential Exposure to Plasticizers and Its Possible Role in the Pathogenesis of Asthma

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The plasticizer di(2-ethylhexyl) phthalate (DEHP) is widely used in building materials. DEHP is identified as the major plasticizer exposure in dwellings. We provide evidence that inhalation exposure to DEHP as aerosols adsorbed to particulate matter is as important, or more important, than vapor phase exposure. The particulate inhalation exposure to DEHP is considered to be significant due to its low clearance and extensive penetration into the pulmonary region. DEHP is capable of creating high local concentrations in the airways at the deposition site with subsequent local effects. The proposed mechanism of effect states that mono(2-ethylhexyl) phthalate (MEHP), the primary hydrolysis product of DEHP, mimics the inducing prostaglandins (PG) PGD_2, 9α,11βPGF_2, and $PGF_{2\alpha}$, and thromboxanes in the lungs, thereby increasing the risk of inducing inflammation in the airways, which is a characteristic of asthma. *Key words*: asthma, di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate, plasticizer, polyvinyl chloride, prostaglandin, PVC, thromboxane A_2 receptor.
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Industrialization has been accompanied by a notable increase in the occurrence of unnatural substances (xenobiotics). Many of these environmental chemicals decompose very slowly and some of them even have bioaccumulative properties. The exposure to xenobiotics results in an increased risk of detrimental effects to health. The extent of this risk is illustrated by recent discoveries of different xenobiotics exhibiting estrogenlike effects. This might result in lower sperm counts, epididymal cysts, cryptorchidism, and testicular cancer (*1*).

Parallel to this, several studies indicate an increased rate of diagnosed asthma among children during the last 30 years (*2–8*). There has been some controversy whether this increase has been due to a genuine increase in asthma or simply to a change in diagnostic criteria. In several industrialized countries, airway inflammation and bronchial reactivity have been observed, including childhood asthma, which affects from 11 to 20% of all children of school age (*9*). Not only is asthma common but the incidence appears to be steadily increasing; from recent reviews, it seems that the incidence of asthma is doubling every 10–15 years (*2,10*). If this trend is to be halted, it is necessary to determine the causal factors and to understand the underlying mechanisms.

Outdoor as well as indoor pollutants have been suggested as risk factors in the development of asthma. Outdoor air pollution has been declining in certain industrialized countries during the last 10 years (*11*), but contrary to expectation, this has not resulted in a corresponding decline in the incidence of asthma. It is therefore a possibility that indoor pollutants might be of greater importance in the development of asthma than outdoor pollutants. Several studies have shown significant associations between health outcomes and exposure to indoor components like environmental tobacco smoke (*12–15*), NO_2 (*16*), mite allergens (*17–19*), and mold or dampness (*20–24*). Also, other residential exposures such as pet allergens and particles have been suggested to cause health problems (*25,26*).

Inflammation of the airways is an important part of the mechanism of asthma and bronchial reactivity (*27*). Most allergens stimulate production of IgE antibodies that bind to mast cells; with linkage to antigens, the mast cells release inflammatory mediators, causing bronchospasm and mucus production. There also appears to be chemical compounds with a capacity to trigger the inflammation without involving IgE (*28*). Long-term occupational exposure to relatively high levels of chemicals such as formaldehyde (*29*), diisocyanates (*30*), and organic anhydrides (*31*) is known to increase the risk of asthma. Exposure to some of these chemical compounds, such as formaldehyde, may also lead to development of specific airway hypersensitivity (*29,32*). Organic acid anhydrides, commonly used for production of plasticizers in the plastic industry, have been suggested to induce production of IgE antibodies (*31*). Little is known about whether the levels of chemical compounds common in the home environment play any role in the causation of bronchial obstruction and asthma. Plastic interior materials are potential sources of chemicals that may cause airway inflammation and increase the risk of bronchial obstruction and asthma. Recently, exposure to plastic interior surfaces have been shown to increase the risk of developing bronchial obstruction during the first 2 years of life (Jaakkola et al., unpublished data). However, little is known about the active agent(s), exposure route(s), and pathogenesis.

The plasticizer di-(2-ethylhexyl) phthalate (DEHP) is widely used in the production of polyvinyl chloride (PVC) and vinyl chloride resins. DEHP accumulates, to a great extent, in building interior surfaces (*33,34*). Further, mono(2-ethylhexyl) phthalate (MEHP), the primary hydrolysis product of DEHP, has been found to induce bronchial hyperreactivity in rats (*35*). Jaakkola et al. (unpublished data) recently observed an association between plastic interior surfaces and bronchial obstruction and suggested DEHP as a possible active agent, without going into possible mechanisms. The objective of the present study was to identify and quantify the major phthalate inhalation exposure routes in residences. We propose a hypothesis on the role of DEHP in the pathogenesis of asthma.

Materials and Methods

Samples. Materials were selected from the Oslo Birth Cohort Study, which is a study of 3,754 children born in Oslo, Norway, 1992–1993 (*36*). From a total of 372 dwellings with performed site inspections and ventilation measurements, 38 dwellings were selected for another study (*37*), but additional samples of sedimented dust and suspended particulate matter were taken for the present study. Briefly, the criteria for inclusion of the dwellings under study were 1) equal representation of residences with predicted high or low suspended particulate matter concentrations and 2) that the occupiers had not moved or renovated their resi-

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dences since the site inspection in the Oslo Birth Cohort Study. The summer season (June to August) was excluded as a measuring period due to extensive airing. The families were urged not to perform any interventions before the end of collection of samples and suspend any housecleaning procedures for 7 days prior to our scheduled site visit.

Sedimented dust samples. Sedimented dust was collected on Millipore aerosol analysis monitors, type A (Millipore, Bedford, MA). The main filter was a Millipore AP40 integral filter made of borosilicate microfibre glass with a diameter of 24 mm, with 0.8–8 μm pores, and without binder material. A cellulose prefilter pad was used to support the main filter and to facilitate an even airflow over the filter area. The inlet on the monitor housing was equipped with a Teflon tube. The outlet was connected to a conical silicone adapter to fit the tube of a standard vacuum cleaner operating at 500 W. After sampling, the filters were stored in sealed glass containers in a refrigerator until weighing and analysis.

Each sedimented dust sample is a pooled sample from the following locations: sheet of child's bed (1 min sampling), floor in child's bedroom (1 m^2, 1 min sampling), floor in central living room (1 m^2, 1 min sampling), and on top of shelves in central living room (0.5 m^2, 0.5 min sampling).

Suspended particulate matter samples. For comparison of sedimented dust and suspended particulate matter with respect to contents of phthalates, 6 residences were randomly selected out of the total of 38 for additional sampling of suspended particulate matter. The families were urged not to perform any interventions before the end of the 7-day sampling period and not to perform any housecleaning procedures during sampling.

Suspended particulate matter was collected on Millipore MHTSO25AC filters (Millipore). The main filter was made of polycarbonate, with a diameter of 37 mm and with 0.4 μm pores. A cellulose prefilter pad was used to support the main filter and to facilitate an even airflow over the filter area.

For quantitative assessment of the sampled material, the polycarbonate filters were weighed before and after sampling on a Mettler MT5 microbalance, with a readability and reproducibility of 1 μg and 0.8 μg, respectively. Before and after sampling, the filters were maintained in a controlled environment (temperature, 22°C; relative humidity, 50%) for at least 12 hr prior to weighing.

All samples of suspended particulate matter were collected with the full opening in the filter cassettes directed approximately 45° downward. From each dwelling, two parallel samples were collected in the same position in the central living room, 1.1 m above the floor and above the table allocated to the most frequently used sitting group. The field monitors were connected by silicone tubes to net-operated high flow pumps (Dymax 30; Charles Austen Ltd., Surrey, U.K.) with an initial capacity of 5.0 l/min without external resistance. The actual flows were measured on site at the start and stop of each measurement by a flowmeter.

Analysis of phthalate esters adsorbed to sedimented dust and suspended particulate matter. After weighing, the sedimented dust samples were transferred from the filter units to 5 ml glass vials with Teflon-lined screw caps. Depending on the sample size, 0.5–2.0 ml of methanol (1.0 ml in most cases) was added. The mixture was thoroughly stirred with a glass rod and left for extraction without stirring at room temperature for 24–48 hr; it was then stirred again and centrifuged. The clear supernatant was analyzed with gas chromatograpy-mass spectrometry (GC-MS).

The samples of suspended particulate matter, in the form of two filters with the adherent fine dust from each of the six residences, were extracted with 0.5 ml of methanol for 24–48 hr in the same type of vials used for sedimented dust. The vials were placed horizontally to ensure wetting of the dust filters.

The sedimented dust samples, left suspended in methanol after extraction as described above, were further suspended in water (50 ml) with shaking. After a few minutes of sedimentation of dense particles (sand, soil, etc.), as much as possible of the remaining suspension of organic material was removed. The process was repeated 10 times. The inorganic fraction obtained was isolated on a preweighed membrane filter, dried in an oven at 40°C for 30 min, and weighed.

The gas chromatograph (Hewlett-Packard 5890; Hewlett-Packard, Palo Alto, CA) was equipped with a standard split/splitless injector and coupled to a VG Trio-2 quadrupole mass spectrometer. The column used was a 30 m × 0.31 mm inner diameter DB-5 fused silica column (J&W Scientific) with a film thickness of 1 μm. Helium was the carrier gas, with a flow rate of about 2 ml/min. The oven temperature was 100°C for 2.5 min, followed by a rise of 10°C/min to 300°C. The final temperature was held for 8 min. The injector and transfer line temperatures were 275°C. Splitless injections of 1 μl of samples and standards were performed with the splitflow closed for 1 min, and with the aid of the solvent flush technique, in which the syringe needle is filled with solvent (methanol) followed by an air plug and the sample. After a solvent delay time of 2.5 min, the mass spectrometer was set to scan full spectra in the positive ion electron impact mode in the mass range m/z 20–350 at a speed of 1 scan/sec. Data were acquired on a personal computer with the Labbase software (VG, Mancester, U.K.).

Results

The weight of the 38 sedimented dust samples ranged from 40 to 609 mg (median = 147 mg). Inspection of the filters revealed the presence of varying, and often substantial, amounts of large particles (sand, soil, etc.), which could bias the results. The weight of the organic fraction, determined by separating and weighing the inorganic fractions, ranged from 27 to 378 mg (median = 119 mg), accounting for 41–92% (mean = 76%) of the total sedimented dust. The accumulated weight of the six parallel suspended particulate matter samples ranged from 838 to 2655 μg (median = 1534 μg).

Chromatographic traces from the analysis of the methanol extract of a sedimented dust sample are shown in Figure 1. The total ion current chromatogram, lower trace, shows that the extract is not very complex. The major constituents are the phthalate esters and a number of compounds of supposed biological (human) origin, including fatty acids and the triterpenoid hydrocarbon squalene, which is secreted from human skin at a rate of 250 mg/day in adults (*38*). The composition of the extracts is qualitatively rather constant. See Figure 1 for a list of common constituents.

The quantification of the phthalate esters was based on mass chromatograms of their characteristic m/z 149 ion peaks (Fig. 1A). The amounts of the three most prominent esters, dibutyl, benzyl butyl, and di(2-ethylhexyl), were determined with the aid of external standard calibrations with four solutions in methanol in the concentration range 2–200 ppm (μg/ml). With the use of mean values from two to five injections of each standard solution, very good linear calibration lines were obtained (r^2 values of 0.9998 or better), but standard deviations were rather large, about 25%. The generally minor amounts of diethyl and diisobutyl esters were quantified with the calibration for the dibutyl ester. The heavier phthalates, appearing to be a mixture of isomeric dinonyl esters, were present in a few of the samples. These were quantified with di(2-ethylhexyl) ester as standard.

The extraction solvent, methanol, seems to be adequate in the sense that reextraction of a sedimented dust sample for 24 hr

showed that all methanol-extractable phthalate ester material had been removed in the first extraction. Further validation of the method included blind analyses of filters and housings. The extract of filters and housing for sedimented dust contained a considerable amount of dioctyl adipate as the only background component, but this ester was not present in the sample extracts. The extract of filters and housing for suspended particulate matter showed no background material except for barely detectable amounts of dibutyl phthalate (DBP) and DEHP, that most likely represent a syringe memory effect.

Table 1 shows the distribution of phthalates adsorbed to sedimented dust. There is a large variation in concentrations of all species of phthalates in sedimented dust. DEHP is the predominant phthalate species in both total dust (64 µg/100 mg) and organic fraction (82 µg/100 mg). In the 38 samples of sedimented dust, DEHP accounts for 32–97% (mean 69%) of the total amounts of phthalates in total dust. DBP, benzyl butyl phthalate (BBP), and the heavier phthalates (mixture of isomeric dinonyl phthalates) each account for approximately 10% of the total amount of phthalates. The amounts of diethyl phthalate (DEP) and diisobutyl phthalate (DIBP) are negligible in most residential sedimented dust samples.

Table 2 shows the distribution of phthalates adsorbed to suspended particulate matter. As in sedimented dust, there is a large variation in concentrations of all species of phthalates in suspended particulate matter. DEHP is the predominant species in the samples of suspended particulate matter (60 µg/100 mg) followed by DBP, BBP, and DEP. In the six samples of suspended particulate matter, DEHP accounts for 52% of total phthalates. DIBP and heavier phthalates were not found in any suspended particulate matter samples. The affinity of phthalates to suspended particulate matter is of the same magnitude as sedimented dust.

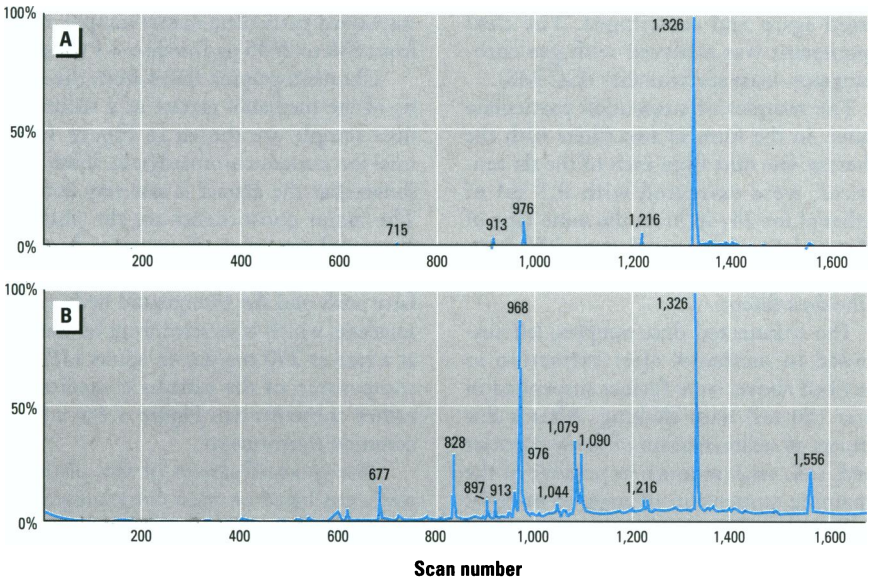

Figure 1. (A) Mass chromatogram (*m/z* 149) and (B) total ion current chromatogram of the methanol extract of a sedimented dust sample. Major components by scan number and name are 677, dodecanoic acid; 715, diethyl phthalate; 828, tetradecanoic acid; 897, pentadecanoic acid; 913, diisobutyl phthalate; 968, hexadecanoic acid; 976, dibutyl phthalate; 1079, octadecenoic acid; 1090, octadecanoic acid; 1216, benzyl butyl phthalate; 1326, di(2-ethylhexyl) phthalate; 1556, squalene.

Table 1. Phthalate concentrations adsorbed to sedimented dust (total and organic fraction) in 38 dwellings in Oslo, Norway

	Mean		Range	
Phthalate species	Total dust	Organic fraction	Total dust	Organic fraction
Dibutyl phthalate	10	12	1–103	1–117
Benzyl butyl phthalate	11	14	0–44	0–48
Di(2-ethylhexyl) phthalate	64	82	10–161	11–210
Diethyl phthalate	1	1	0–11	0–17
Diisobutyl phthalate	1	1	0–30	0–45
Heavier phthalates[a]	10	12	0–138	0–161
Total phthalate	96	123	13–292	17–327

Values are given in µg/100 mg sedimented dust.
[a]Mixture of isomeric dinonyl phthalates.

A normal active adult inhales 14 m^3 of air in 24 hr (*39*). With an exposure to suspended particulate matter (PM_{10}) of 90 µg/m^3 [national 8-hr average guideline (*40*)], the particulate exposure to DEHP will be 0.30–1.18 µg/day (mean 0.76 µg/day) according to our measurements of DEHP particle exposure.

As shown in Table 3, a significant correlation was found between the concentrations of the plasticizers DEHP and BBP adsorbed to suspended particulate matter and the corresponding concentrations adsorbed to both total and organic fraction of sedimented dust. For the dominating plasticizer, DEHP, a scatter plot displaying the correlation is presented in Figure 2.

Discussion

Residential exposure to phthalates. DEHP is identified as the major phthalate exposure compound, both in sedimented dust and in suspended particulate matter. On average, DEHP accounts for 69% of the total amount of phthalates adsorbed to sedimented dust and for 52% of suspended particulate matter. A large variation in amounts of phthalates adsorbed to both sedimented dust and suspended particulate matter was found, indicating an equally large variation in exposure. The samples of sedimented dust could be biased by particles from PVC flooring or other materials containing plasticizers. However, this is not likely to be the case for the plasticizers DEHP and BBP because there is a significant correlation between concentration in suspended particulate matter and in sedimented dust samples. This indicates that sedimented dust samples are good surrogates of suspended particulate matter samples with respect to adsorbed DEHP and BBP.

Because of very slow volatilization of DEHP from plastic products, the airborne human exposure is estimated to be 0.4 µg/day (*41*). In these calculations, only the vapor phase exposure is considered, which leads to underestimation of real exposure. In this study we have shown that the residential

Table 2. Phthalate concentrations adsorbed to suspended particulate matter in six dwellings in Oslo, Norway

Phthalate species	Mean ± SD	Range
Dibutyl phthalate	37 ± 22	13–69
Benzyl butyl phthalate	14 ± 30	0–75
Di(2-ethylhexyl) phthalate	60 ± 30	24–94
Diethyl phthalate	8 ± 9	0–24
Diisobutyl phthalate	0 ± 0	0–0
Heavier phthalates[a]	0 ± 0	0–0
Total phthalate	118 ± 63	45–226

SD, standard deviation. Values are given in µg/100 mg suspended particulate matter.
[a]Mixture of isomeric dinonyl phthalates.

exposure of DEHP adsorbed to suspended particulate matter is one- to threefold the estimated daily vapor phase exposure in the general population. This estimation must be considered as conservative because, under normal housing activities, the PM_{10} fraction of total suspended particles may be only about 60%, due to strong resuspension effects of housing activities (*42*). The calculated ratio of one- to threefold particulate exposure compared to vapor phase exposure must be interpreted as approximate, due to the very limited data on DEHP vapor phase levels in indoor air and because we have not performed parallel measurements of the vapor phase concentrations in the indoor environments under study. Infants per kilo weight have a respirational volume twice as large as adults (*39*) and spend most of the time indoors and, in particular, in bedrooms. Children's bedrooms are often small rooms and with one door and one window only. A small room has a higher wall surface to room volume ratio than a large room; thus, the emission from building materials is highest in a small room. Hence, small children are subject to the highest exposure risk. The particulate inhalation exposure to DEHP is considered to be the most significant because of its low clearance and extensive penetration into the pulmonary region and its capability of creating high local concentrations in the airways at the deposition site, with subsequent local effects; the sum of these local effects could become severe. However, we found no studies regarding such local high concentrations.

Exposure to DEHP and possible mechanisms involved in the pathogenesis of asthma. The plasticizer DEHP is widely used in the production of PVC and vinyl chloride resins and accumulates to a great extent in building interior surfaces (*33,34*). The use of PVC flooring in Norway is estimated to be 8 million m^2/year (*34*). This is about four times the per capita use of PVC flooring in Europe as a whole (*34*). DEHP may constitute 40% or more of the plastic product. In industrialized countries, approximately 50% of phthalate esters are estimated to accumulate in buildings as compounds in different building and interior materials (*33*). The increased use of phthalate esters has occurred simultaneously with the reported increase in asthma. However, we found no studies regarding time trends in indoor air exposure to DEHP. The causality in this correlation is therefore unknown.

The development of bronchial hyperreactivity/hypersensitivity and asthma is assumed to be attributable to several factors. The inflammatory process, with epithelium damage and edema, is a suggested contributor in pathogenesis (*43,44*). The inflammatory mediators prostaglandin (PG) D_2 and thromboxanes have a variety of effects on target cells in the airways, which may be relevant in the etiology of asthma. PGD_2 induces a pronounced contraction of human airway smooth muscle and increases the bronchial sensitivity (*43,45,46*). This is supported by evidence that PGD_2 is released *in vivo* in the human lower respiratory tract after acute allergic challenge (*47*). PGD_2 is preferentially metabolized by the human lung to 9α,11β prostaglandin F_2 (9α,11βPGF_2), which also has bronchoconstrictive characteristics. Although 9α,11βPGF_2 is less potent than PGD_2, it is assumed to have a larger effect in the lung because of slower degradation (*48*). All the contractive prostaglandins and thromboxanes react via an apparent single thromboxane (TP) A_2 receptor (*49,50*). Other TP receptor subtypes may exist, which could explain why the TP receptor is not very selective; however, strong evidence supports the existence of a single form of the TP receptor in both platelets and smooth muscle cells (*5*). Receptor binding is suggested as contributing actively to the pathogenesis of bronchial asthma (*7,45,46*).

We propose that the increase in asthma is due to contributory factors of environmental chemicals in general, and specifically DEHP through its primary hydrolysis product MEHP, which affects the bronchial contracting receptors and thereby generates a hyperreactive condition in the lungs. This will increase the risk of a pathological development in addition to aggravation of the effects of other environmental agents.

The bronchial contracting prostaglandin receptors are particularly sensitive to environmental chemicals for the following reasons:

- Under normal conditions the inflammatory mediators PGD_2 and 9α,11βPGF_2 are rapidly metabolized by specialized enzymes in lung tissues. MEHP is not equally suited to be metabolized by these enzymes and, in consequence, will affect the receptors over longer periods. This indicates that environmental chemicals are not required to be particularly potent bronchial contractors in order to exhibit a marked effect on lung tissues.
- The bronchial contracting prostaglandin receptors are suggested to be relatively unspecific.

As shown in Figure 3, there is a structural similarity between DEHP, MEHP, and the prostaglandins, regarding both the molecular size and ring structure. This favors DEHP and MEHP as the possible active species in the recent observed association between PVC interior surface materials and bronchial obstruction (Jaakkola et al., unpublished data). The bronchial contracting prostaglandin receptors are suggested to be relatively unspecific. Recently, the TP receptor has been analyzed and the amino acid sequence and three-dimensional structure model has been elucidated (*6*). The receptor is composed of a hydrophobic pocket embedded in the cell membrane and a hydrophilic extracellular part. When the receptor is stimulated by a prostaglandin, it is assumed that the ring structure is embedded in the hydrophobic pocket. It is probably the stimulation of the hydrophobic pocket, which elicits the signal transmission. Xenobiotics that fit into this pocket could probably produce a response. Among xenobiotics there are some obvious candidates, e.g., phthalates, adiapates, alkylbenzens, nonylphenols, and others.

The lung toxicological information for DEHP indicates that intravenous administration of DEHP and its hydrolysis product MEHP accumulate in the lungs of rats (*51*). Intravenous injection of DEHP in rats will result in tracheal bleeding, tracheal inflammation, and rapid death (*52*). Based

Table 3. Spearman correlation coefficients between plasticizer concentrations in sedimented dust (total and organic fraction) and suspended particulate matter

Plasticizer species	Total sedimented dust vs. suspended particulate matter	Organic fraction vs. suspended particulate matter
Dibutyl phthalate	0.03 (p = 0.96)	-0.09 (p = 0.87)
Benzyl butyl phthalate	0.85 (p<0.05)	0.85 (p<0.05)
Di(2-ethylhexyl) phthalate	0.83 (p<0.05)	0.83 (p<0.05)
Diethyl phthalate	0.13 (p = 0.80)	0.13 (p = 0.80)
Diisobutyl phthalate	0.66 (p = 0.16)	0.43 (p = 0.40)

Two-tailed significance levels are given in parentheses.

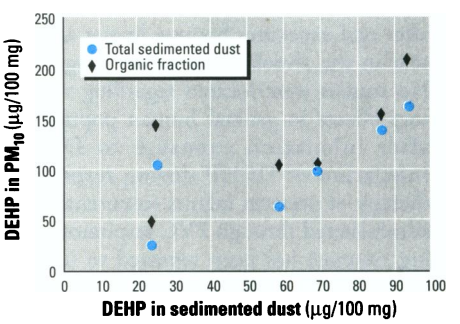

Figure 2. Correlation between the amount of di(2-ethylhexyl) phthalate (DEHP) in suspended particulate matter (PM_{10}) and the DEHP concentrations in total sedimented house dust and the organic fraction of sedimented house dust.

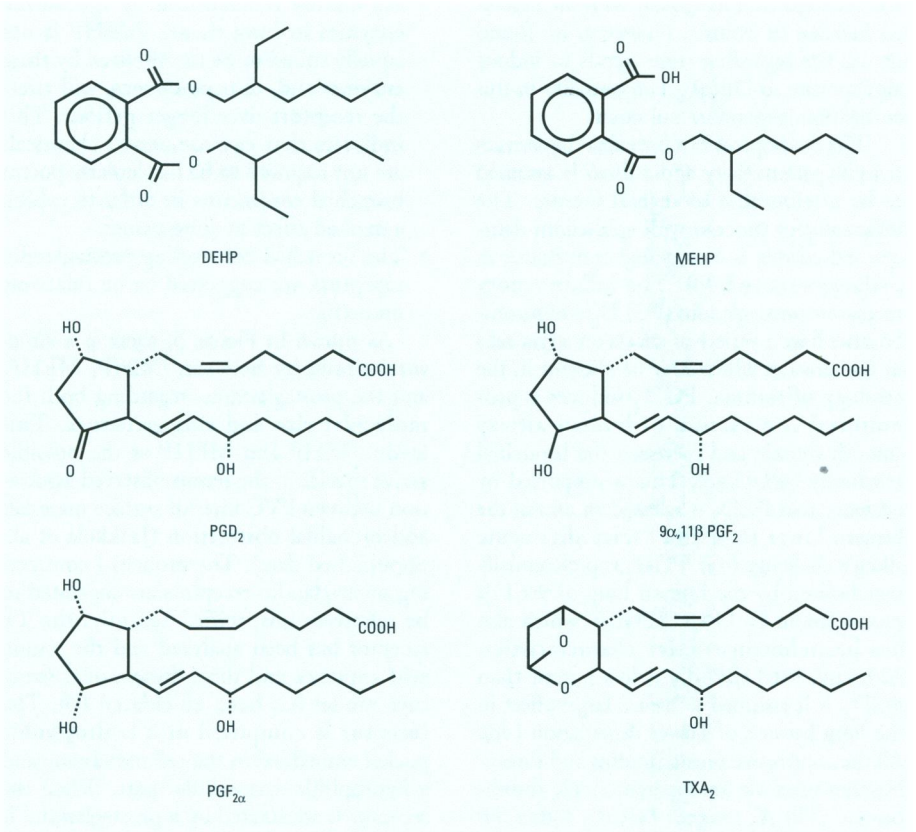

Figure 3. Structural similarities between proposed xenobiotics (DEHP and MEHP) and inflammation prominent mediators as prostaglandins and thromboxanes. Abbreviations: DEHP, di(2-ethylhexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; PGD_2, prostaglandin D_2; 9α,11β PGF_2, 9α,11β prostaglandin F_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; TXA_2, thromboxane A_2.

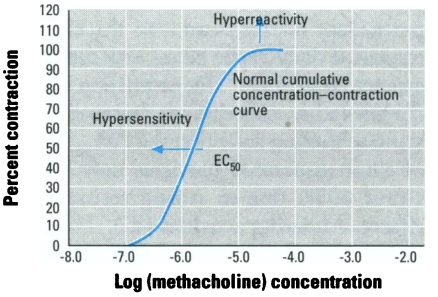

Figure 4. Principal normal cumulative log dose–response curve, illustrated as percent contraction of tracheal tissue due to challenge with methacoline. Hyperreactivity is defined as an abnormal increase in the maximum response obtained by an increase in the concentration of the active agent, while hypersensitivity implies a normal response to a lower than normal concentration of the challenging agent (*54*). EC_{50}, median effective concentration.

on data from both human and animal studies, the metabolism of DEHP involves a complex series of reactions with the production of 30 or more metabolites, but the primary metabolite is MEHP (*41*). DEHP is partially hydrolyzed to MEHP, followed by oxidation of the remaining side chain (*53*). No data were located regarding the metabolites produced in humans or animals after inhalation exposure to DEHP, but the metabolism following this route of exposure is expected to be similar to that after oral exposures because lipases are present in the alveolar cells of the lungs (*41*). No studies were located regarding respiratory effects in general human populations after inhalation exposure to DEHP. Inhalation of DEHP during respiration therapy of preterm infants (unintentionally administered through PVC respiratory tubing systems) has been reported to increase the risk of bronchial asthma (*53*). This must be considered as an almost pure vapor phase exposure to DEHP due to the highly effective filters in the respiratory tubing system. The pharmacological effects of DEHP and its metabolites MEHP and phthalic acid on rat muscarinic receptor response have also been studied (*35*). Methacholine dose–response curves of rat tracheal tissue were not influenced by DEHP or phthalic acid (up to concentrations of 1 mM), but incubation with 0.1 mM MEHP induced a significant increase in bronchial sensitivity. The absence of similar effects of DEHP is explained by its high lipophilicity, rendering it unable to reach its site(s) of action. With exposure to DEHP particulate matter in concentrations found in the present study, the effective MEHP dose of 0.1 mM in rats (*35*) is considered likely to occur locally in human tracheal tissue too.

Asthma becomes manifest through pulmonary inflammation and increased nonspecific reactivity to several stimuli. Clinically, this hyperreactivity is observed by stimulating the lungs with nonspecific stimuli such as histamine or methacholine in a concentration that produces the same bronchial contractive effects. In a clinical sense, bronchial hyperreactivity is a consequence of two different phenomena: 1) hypersensitivity implies a normal response to lower than normal concentrations of the challenging agent, corresponding to a left parallel shift in the log dose–response curve; and 2) hyperreactivity is defined as an abnormal increase in the maximum response obtained by an increase in the concentration of the active agent (steeping in the slope of the log dose–response curve and increase of amplitude) (*54*). This is illustrated in Figure 4.

Doelman et al. (*51*) have shown that MEHP, in *in vitro* experiments with rat tracheal tissue, induces a dose-dependent decrease in log median effective concentration (EC_{50}) for methacholine curves, in other words, a dose-dependent increase in respiratory sensitivity. Doelman et al. (*51*) also showed that the level of respiratory hyperreactivity declined with relatively high concentrations of MEHP. Both DEHP and MEHP have been reported to be effective inhibitors of the protein kinase C enzyme, which is involved in both the muscarinic and TP receptor transduction pathways (*5,51,55*). This is illustrated in Figure 5.

To investigate whether the hypersensitivity following MEHP exposure is due to protein kinase C, Doelman et al. (*51*) tested the effects of the known specific protein kinase C inhibitor 1(5-isoquinolinylsulphonyl)2-methylpiperazine (H7) on the methacholine dose–response curve of the rat trachea. Incubation with H7 induced a decrease in maximal effect, i.e., a decrease in hyperreactivity, but did not influence the hypersensitivity. The authors concluded that a decrease in log EC_{50} following exposure to MEHP was not due to protein kinase C inhibition, whereas the reduction of the maximum effect (hyperreactivity) with high levels of MEHP might be.

These results support the proposed mechanisms involved with DEHP exposure. If our hypothesis about mechanisms is

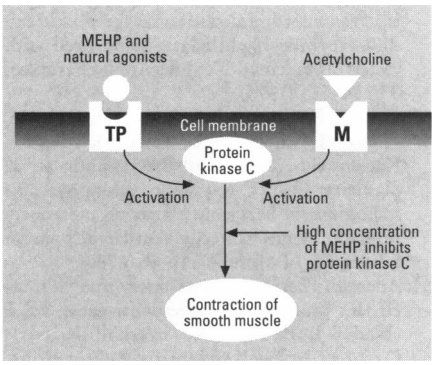

Figure 5. The general contraction as described by Doelman et al. (*51*) is due to stimulation of the muscarine receptor (M) by acetylcholine. An additional contraction effect is proposed by mono(2-ethylhexyl) phthalate (MEHP) stimulation of thromboxane A_2 receptor (TP). Both receptors are known to activate protein kinase C.

correct, MEHP exposure will result in a dose-dependent stimulation of the TP receptor leading to increased hypersensitivity. With higher concentrations of MEHP, it is possible that an inhibition of protein kinase C will result in a corresponding decline in hyperreactivity. A single receptor may be involved in the parallel left shift of the log EC_{50} curve after MEHP exposure. A decline in the hyperreactivity is most often associated with a restricted signal transfer from the receptor to the effector (*54*). A final proof of the proposed hypothesis could be achieved by preventing the MEHP-induced hypersensitivity by adding a TP receptor antagonist such as BAY u3405, which is a selective competitive antagonist (*56*).

A likely modifier in the relationship between inhalation exposure to DEHP and asthma is dietary intake of omega-3 polyunsaturated fatty acids (PUFA). Several studies have shown dietary intake of PUFA to have anti-inflammatory properties (*57,58*). The anti-inflammatory effect of PUFA is due to the fact that the inflammatory mediators thromboxane A_3 (TXA_3) and leukotriene B_5 (LTB_5) synthesized from PUFA give rise to lower biological effects than corresponding mediators (TXA_2 and LTB_4) derived from arachidonic acid (*59*). If the dietary intake of PUFA is insufficient, then cellular response will increase and may therefore contribute to increased respiratory hyperreactivity. We have found that exposure to plasticized materials in residences increases the risk of bronchial obstruction in young children (Jaakkola et al., unpublished data). Further analysis reveals that dietary intake of PUFA is a strong modifier which supports the hypothesis of DEHP exposure as the active agent in the association between exposure to plastisized materials and bronchial obstruction in young children. Further, it supports the proposed mechanisms of effect as described in this study.

In conclusion, we have shown that suspended particle exposure to the plasticizer DEHP is one- to threefold higher than the estimated vapor phase exposure and that previous estimates of daily airborne intake are underestimated because the particle fraction is overlooked. Further, the possible mechanisms of respiratory effects by inhalation exposure to DEHP has been elaborated. DEHP is primarily hydrolyzed to MEHP, followed by oxidation of the remaining side chain. The proposed mechanism of effect states that MEHP mimics the inducing prostaglandins PGD_2, $9\alpha,11\beta$ PGF_2, and $PGF_{2\alpha}$ and thromboxanes in the lungs, thereby increasing the risk of inducing inflammation in the airways, which is a characteristic feature of asthma.

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