Phthalate Exposure and Breast Cancer Incidence: A Danish Nationwide Cohort Study

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PURPOSE Phthalate exposure is ubiquitous and especially high among users of drug products formulated with phthalates. Some phthalates mimic estradiol and may promote breast cancer. Existing epidemiologic studies on this topic are small, mostly not prospective, and have given inconsistent results. We estimated associations between longitudinal phthalate exposures and breast cancer risk in a Danish nationwide cohort, using redeemed prescriptions for phthalate-containing drug products to measure exposure.

METHODS We ascertained the phthalate content of drugs marketed in Denmark using an internal Danish Medicines Agency ingredient database. We enrolled a Danish nationwide cohort of 1.12 million women at risk for a first cancer diagnosis on January 1, 2005. By combining drug ingredient data with the Danish National Prescription registry, we characterized annual, cumulative phthalate exposure through redeemed prescriptions. We then fit multivariable Cox regression models to estimate associations between phthalate exposures and incident invasive breast carcinoma according to tumor estrogen receptor status.

RESULTS Over 9.99 million woman-years of follow-up, most phthalate exposures were not associated with breast cancer incidence. High-level dibutyl phthalate exposure ($\geq$ 10,000 cumulative mg) was associated with an approximately two-fold increase in the rate of estrogen receptor–positive breast cancer (hazard ratio, 1.9; 95% CI, 1.1 to 3.5), consistent with in vitro evidence for an estrogenic effect of this compound. Lower levels of dibutyl phthalate exposure were not associated with breast cancer incidence.

CONCLUSION Our results suggest that women should avoid high-level exposure to dibutyl phthalate, such as through long-term treatment with pharmaceuticals formulated with dibutyl phthalate.

INTRODUCTION

Phthalates are used in many consumer goods, including medical supplies, food containers, cosmetics, and toys.[1] Phthalates are also used in pharmaceutical capsules to impart delayed- or extended-release properties.[2] Phthalates are not covalently bound to other constituents and readily leach out of products, leading to human exposure.[3] Studies show that most tested adults in developed regions have measurable urinary phthalate metabolites.[1,4-7] Exposure is particularly high among users of phthalate-containing medications.[8,9] For example, users of mesalamine had approximately 60-fold higher levels of urinary mono-butyl phthalate—a metabolite of dibutyl phthalate (DBP)—than nonusers who were exposed only through environmental sources.[8,10] Such widespread exposure[11,12] has raised concerns about the health consequences of phthalates.[3]

Phthalates are potential endocrine disruptors—exogenous compounds that mimic hormones and may

therefore affect fertility,[13-15] fetal/child development,[3,16-18] and some cancers.[3,19] Preclinical evidence suggests that some phthalates promote breast tumor growth through estrogen receptor (ER) signaling.[20-25] Estrogen-independent breast cancer promotion mechanisms have also been reported for phthalates,[21,26-29] as have antiestrogenic effects.[23]

Epidemiologic evidence is inconsistent regarding the effect of phthalates on breast cancer incidence. Existing studies relied on measurement of urinary metabolites as a proxy for exposure to the parent compounds hypothesized to affect health. A 2010 case-control study measured nine urinary metabolites of six different phthalates in 233 Mexican patients with breast cancer and 221 controls.[30] The authors reported a potentially causal association with a diethyl phthalate (DEP) metabolite and potentially protective associations with metabolites of butyl benzyl phthalate (BBP) and dioctyl phthalate (DOP).[30] Breast cancer was not notably associated with metabolites of DBP, di-isobutyl phthalate, or diethylhexyl phthalate

(DEHP).[30] In contrast, a case-control study of Alaskan native women found a potentially causal association for monoethylhexyl phthalate (a DEHP metabolite),[31] and a cross-sectional study from the U.S. National Health and Nutrition Examination Survey found no notable breast cancer associations with metabolites representing di-isobutyl phthalate, DEP, DOP, BBP, and DEHP.[32] Most recently, a case-control study of postmenopausal Women's Health Initiative participants measured urinary metabolites at multiple time points in prospectively collected samples. The authors reported no positive associations between phthalates and breast cancer overall.[33]

Given the ubiquity of phthalate exposure and the inconsistent epidemiologic evidence, we measured associations between prospectively recorded phthalate exposures and breast cancer incidence in a Danish nationwide cohort. To accomplish this, we capitalized on the documented high-level phthalate exposure through use of certain medications. On the basis of preclinical evidence, we hypothesized that exposure to DBP, an estrogenic phthalate that is also a common medication excipient,[11,12] would be positively associated with ER-positive breast cancer.

METHODS

Danish Population-Based Registries

Denmark maintains extensive population-based registries that capture information on health, employment, vital status, and emigration.[34] We enumerated a cohort of women at risk for breast cancer using data from several independent registries, all of which were linked at the individual level using the CPR number, a 10-digit identifier assigned to legal residents of Denmark.

Phthalate Content of Medications

The Danish Medicines Agency maintains a database of pharmaceuticals included in the Danish formulary. For each marketed drug, it records the Nordic Article Number (also called a VNR code—a unique identifier assigned to medicinal agents from different manufacturers), the Anatomic Therapeutic Chemical (ATC) code, dates of market entry and removal, and both active and inactive ingredients. Ingredient data include the mass of each component per medication unit (eg, milligrams per capsule). Although the database covers drugs marketed since 1995, excipient data are complete since 2005. We searched ingredient fields for phthalate-related text strings in both Danish and English and prepared a database of phthalate-containing oral medications. Products without an ATC code (ie, herbal products and dietary supplements) were excluded.

We merged the drug database with the Danish National Prescription Registry (DNPR).[35] For each redeemed prescription, the DNPR records the patient's CPR number, transaction date, drug filled (ATC and VNR codes), and quantity dispensed. Information is updated daily and used by the Danish government to partially refund out-of-pocket medication costs.

Source Population and Data Collection

Our source population was all female residents of Denmark who were alive, without a cancer history, and unexposed to phthalate-containing medications between January 1, 1995, and January 1, 2005. We identified this population by linking the Danish Civil Registry, the Danish Cancer Registry, and the augmented DNPR.

Definitions of Analytic Variables

Follow-up began on January 1, 2005. We ascertained incident invasive breast cancer cases from the Danish Cancer Registry.[36,37] We classified cases as ER-positive or ER-negative by merging tumor data from the Danish Breast Cancer Group Registry.[38,39] Vital status came from the Danish Civil Registry.[40,41] We calculated individuals' person-time as the days elapsed between baseline and the first of breast cancer diagnosis, other cancer diagnosis, death, emigration, or the end of follow-up on December 31, 2015. We calculated the phthalate content of each filled prescription by multiplying the mass of phthalate per capsule by the fill quantity. We characterized phthalate exposures as the cumulative milligrams of cellulose acetate phthalate (CAP), DEP, DBP, hypromellose phthalate (HPMCP), or polyvinyl acetate phthalate (PVAP) contained in all prescriptions filled by a patient during each year of follow-up. Categories for cumulative phthalate exposures were based on the observed distribution of continuous values in the final year of follow-up. Cumulative exposures to DBP, CAP, and HPMCP were categorized as no exposure, 1 to 249 mg, 250 to 999 mg, 1,000 to 9,999 mg, and 10,000 mg or more. Cumulative exposure to DEP was categorized as unexposed, 1 to 9 mg, 10 to 99 mg, and 100 mg or more. PVAP exposure was rare and therefore modeled as a dichotomous variable (unexposed v any exposure [range, 1.3 to 682 cumulative grams]).

We defined the following set of potential confounders. Age was defined at baseline; menopausal status was imputed as pre- or postmenopausal on the basis of age younger than 55 years or age 55 years or older, respectively; other phthalate exposures were classified dichotomously on the basis of any versus no exposure during follow-up (eg, when modeling DBP exposure, we adjusted for exposure to CAP, DEP, PVAP, and HPMCP); medication exposures were classified dichotomously for each year of follow-up on the basis of the fifth level of the ATC code (eg, diclofenac, erythromycin, and mesalamine); exposures to cardiac glycosides,[42] hormone therapy,[43] aspirin,[44] oral contraceptives,[45] and statins[46] were positive if a patient redeemed one or more relevant prescriptions in the year before follow-up began; and the Charlson comorbidity index (CCI) was calculated for each study participant from baseline diagnoses.[47] We could not characterize a complete reproductive history but ascertained parity

(nulliparous, one child, two children, or three or more children) from the Danish Medical Birth Register[48] for women age 45 years or older in 2005 (ie, women whose reproductive years were expected to be covered by the Register).

Statistical Analysis

We tabulated the number of medicinal products contributing to phthalate exposures in the cohort and calculated the median and range of phthalate masses in those products. We compared participant characteristics according to dichotomized phthalate status over follow-up. We then fit cause-specific Cox regression models to estimate associations between phthalate exposures and breast cancer incidence. Cumulative milligrams of phthalate exposure was modeled as a time-varying factor variable (using the categories defined above), updated yearly. We specified a 1-year exposure lag to avoid reverse causation bias and because proximate exposure is unlikely to affect risk. To account for competing risks, we censored follow-up upon diagnosis with a nonbreast malignancy (except non-melanoma skin cancer), death, emigration from Denmark, or reaching the end of available follow-up on December 31, 2015. We modeled incidence of ER-positive and ER-negative disease by fitting cause-specific Cox regression models in which ER-negative and ER-positive disease, respectively, was included in the set of competing events.[49] We evaluated modification of the hazard ratio (HR) by

TABLE 1. Medications Contributing to Phthalate Exposures, Denmark, 2005 to 2015

Medication Name	ATC Code	No. of Unique Drug Products Prescribed to the Cohort					
		CAP	DBP	DEP	HPMCP	PVAP	Phthalate Free
Bisacodyl	A06AB02		3	5	5		9
Budesonide	A07EA06		2				27
Clarithromycin	J01FA09				9		48
Diclofenac	M01AB05	2	3	2			144
Diclofenac, combination	M01AB55	2		2			16
Dipyridamole	B01AC07				3		17
Duloxetine	N06AX21				9		39
Erythromycin	J01FA01			15	15		26
Esomeprazole	A02BC05			5			172
Fluoxetine	N06AB03			2			48
Galantamine	N06DA04			3			50
Ibuprofen	M01AE01			13			120
Lithium	N05AN01		1				1
Mesalamine	A07EC02		5				63
Mianserin	N06AX03			14			24
Multi-enzyme	A09AA02	2	2	2	13		47
Mycophenolic acid	L04AA06				1		14
Naproxen	M01AE02	8		8			24
Platelet inhibitors, combination	B01AC30				2		9
Pentoxyverine	R05DB05	1					2
Propantheline	A03AB05	2					2
Rabeprazole	A02BC04				7		8
Sulfasalazine	A07EC01	3					15
Theophylline	R03DA04	6		2			6
Valproic acid	N03AG01	3				9	40
Verapamil	C08DA01			13			56
Total No. of products		29	16	86	64	9	1,057
Milligrams of phthalate per capsule, median (minimum-maximum)		24 (0.35-60)	1.9 (0.38-8.2)	0.45 (0.003-10)	4.7 (4.5-150)	47 (18-69)	N/A

Abbreviations: CAP, cellulose acetate phthalate; DBP, dibutyl phthalate; DEP, diethyl phthalate; HPMCP, hydroxypropyl methylcellulose phthalate; N/A, not applicable; PVAP, polyvinyl acetate phthalate.

TABLE 2. Characteristics of the Cohort According to Medication-Associated Phthalate Exposure Status: Women Living in Denmark and at Risk for a First Cancer Diagnosis, 2005 to 2015 (N = 1,122,042)

Characteristic	No Phthalate Exposure (n = 960,305; 86%)	Any Phthalate Exposure (n = 161,737; 14%)
Age in 2005, median (q1-q3)	51 (40-63)	58 (44-72)
Charlson comorbidity index		
0, no comorbidity	863,970 (90)	130,080 (80)
1, mild comorbidity	71,725 (7.5)	22,627 (14)
2, moderate comorbidity	17,082 (1.8)	6,070 (3.8)
3, severe comorbidity	7,528 (0.8)	2,960 (1.8)
Medication exposures		
Bisacodyl	14,058 (1.5)	24,555 (15)
Budesonide	4,258 (0.4)	1,810 (1.1)
Clarithromycin	54,797 (5.7)	17,648 (11)
Diclofenac	189,095 (20)	47,515 (29)
Diclofenac, combinations	11,019 (1.2)	18,882 (12)
Dipyridamole	1,569 (0.2)	15,894 (9.8)
Duloxetine	9,712 (1.0)	7,885 (4.9)
Erythromycin	30,298 (3.2)	42,494 (26)
Esomeprazole	64,252 (6.7)	22,198 (14)
Fluoxetine	10,262 (1.1)	3,599 (2.2)
Galantamine	443 (0.1)	1,277 (0.8)
Ibuprofen	456,223 (48)	99,455 (61)
Lithium	1,045 (0.1)	2,481 (1.5)
Mesalamine	4,261 (0.4)	2,013 (1.2)
Mianserin	13,440 (1.4)	12,201 (8.2)
Multi-enzyme	842 (0.1)	857 (0.5)
Mycophenolic acid	61 (0.01)	26 (0.02)
Naproxen	36,792 (3.8)	13,114 (8.1)
Platelet inhibitors, combination	482 (0.05)	7,643 (4.7)
Pentoxyverine	29 (< 0.01)	54 (0.03)
Propantheline	395 (0.04)	787 (0.5)
Rabeprazole	366 (0.04)	1,181 (0.7)
Sulfasalazine	1,440 (0.2)	6,333 (3.9)
Theophylline	425 (0.04)	1,837 (1.1)
Valproic acid	2,898 (0.3)	3,855 (2.4)
Verapamil	9,596 (1.0)	5,958 (3.7)
Comedication use		
Oral contraceptives	106,482 (11)	13,793 (8.5)
Hormone therapy	98,313 (10)	23,984 (15)
Cardiac glycosides	9,625 (1.0)	2,998 (1.9)
Statins	45,781 (4.8)	13,677 (8.5)
Aspirin	60,146 (6.3)	20,848 (13)

NOTE. Data presented as No. (%) unless otherwise noted.

imputed menopausal status by fitting models within strata of women who were premenopausal or postmenopausal throughout their entire follow-up. We visualized heterogeneity of associations between these strata by plotting and visually evaluating CI functions.[50] We verified the proportional hazards assumption by evaluating interactions between fixed exposures and the logarithm of person-time.[49]

Main regression models were adjusted for age (continuous), postmenopausal status (dichotomous, time-varying), exposure to other phthalates (dichotomous), exposure to drug substances contributing to phthalate exposure (dichotomous, time-varying), CCI (factor variable), and baseline use of cardiac glycosides, hormone therapy, oral contraceptives, aspirin, or statins (each dichotomous). The main model of PVAP exposure was not adjusted for drug substance exposures because only one drug contributed to exposure. Models of ER-positive and ER-negative disease could support adjustment only for age, menopausal status, and drug substance exposures. ER-specific models for PVAP exposure could support adjustment only for age. Models stratified by menopausal status were adjusted only for age and drug substance exposures. Analyses were performed with SAS v.9.4 (SAS Institute, Cary, NC).

Sensitivity Analyses

We evaluated the robustness of our findings in the following sensitivity analyses. First, we modeled associations between DBP exposure and breast cancer among users of lithium and mesalamine, the two medications that contributed the most to DBP exposure (Appendix Fig A1, online only; Appendix Tables A1 and A2, online only). Because lithium has been associated with breast cancer growth in vitro,[51] and because cumulative lithium doses tended to be higher among DBP-exposed women (Appendix Fig A2, online only), we evaluated the impact of adjustment for time-varying, cumulative moles of lithium ion on the DBP/breast cancer association. Second, we modeled associations without an exposure lag and with a longer lag of 2 years.

RESULTS

Phthalates in Medications

We identified 430 unique drug products from 29 medications in the Danish formulary with one or more phthalates in their formulation. Cohort members filled prescriptions for 204 of these products, representing 26 drug substances (Table 1). PVAP exposure was informed only by valproic acid, but all other phthalate exposures were informed by several drug substances. Phthalate content ranged from 3 μg to 150 mg per capsule. All medications with phthalate-containing products were also represented by one or more products (range, 1 to 172 products) with phthalate-free formulations.

Characteristics of the Cohort

Our cohort included 1,122,042 women. During 9.99 million person-years of follow-up (median, 10 years), 27,111 cases

TABLE 3. Associations Between Cumulative Phthalate Exposure and Incident Invasive Breast Cancer, Denmark 2005 to 2015

Exposure	Cases	Person-Years	Age Adjusted		Multivariable*	
			HR	95% CI	HR	95% CI
Dibutyl phthalate, mg						
Unexposed	26,966	10,074,227	1.0	Ref	1.0	Ref
1-249	96	33,441	0.92	0.76 to 1.1	0.94	0.77 to 1.1
250-999	16	6,633	0.77	0.47 to 1.3	0.82	0.50 to 1.4
1,000-9,999	20	10,432	0.72	0.46 to 1.1	0.80	0.51 to 1.3
≥ 10,000	13	2,816	1.8	1.0 to 3.1	2.0	1.1 to 3.6
Diethyl phthalate, mg						
Unexposed	25,013	9,430,056	1.0		1.0	Ref
1-9	336	119,177	1.1	0.95 to 1.2	1.0	0.92 to 1.1
10-99	1,264	421,641	1.0	0.97 to 1.1	1.0	0.95 to 1.1
≥ 100	498	156,675	0.99	0.90 to 1.1	0.98	0.89 to 1.1
Cellulose acetate phthalate, mg						
Unexposed	26,718	9,996,855	1.0	Ref	1.0	Ref
1-249	118	38,218	0.93	0.78 to 1.1	0.88	0.73 to 1.1
250-999	82	28,072	0.92	0.74 to 1.1	0.84	0.67 to 1.1
1,000-9,999	131	42,591	0.98	0.82 to 1.2	0.90	0.75 to 1.1
≥ 10,000	62	21,813	0.99	0.77 to 1.3	0.93	0.70 to 1.3
Hypromellose phthalate, mg						
Unexposed	25,930	9,741,655	1.0	Ref	1.0	Ref
1-249	114	31,975	0.98	0.82 to 1.2	0.97	0.80 to 1.2
250-999	555	186,862	1.0	0.94 to 1.1	0.97	0.88 to 1.1
1,000-9,999	415	137,004	0.93	0.84 to 1.0	0.99	0.88 to 1.1
≥ 10,000	97	30,053	0.83	0.67 to 1.0	0.99	0.78 to 1.3
Polyvinyl acetate phthalate						
Unexposed	27,091	10,120,240	1.0	Ref	1.0	Ref
Any exposure	20	7,309	0.91	0.59 to 1.4	0.93†	0.60 to 1.4

Abbreviation: HR, hazard ratio.

*Adjusted for age, menopausal status, other phthalate exposures, use of potentially confounding comedications (cardiac glycosides, hormone therapy, aspirin, oral contraceptives, and statins), drug substance exposures, and Charlson comorbidity index.

†Adjusted for age, menopausal status, other phthalate exposures, use of potentially confounding comedications (cardiac glycosides, hormone therapy, aspirin, oral contraceptives, and statins), and Charlson comorbidity index.

of invasive breast cancer occurred, 84% of which were ER-positive. Approximately 14% of the cohort (n = 161,737) redeemed prescriptions for phthalate-containing medications. Compared with unexposed patients, phthalate-exposed patients were older and more likely to have comorbid disease, be exposed to the drug substances contributing to phthalate exposure, and have taken oral contraceptives, hormone therapy preparations, cardiac glycosides, statins, and aspirin (Table 2).

Phthalate Exposure and Breast Cancer Risk

We observed near-null associations between DEP, CAP, HPMCP, and PVAP exposures and invasive breast cancer incidence (Table 3). Associations remained near-null in ER-specific models (Table 4). The highest category of cumulative DBP exposure (≥ 10,000 mg) was associated with a two-fold increase in the breast cancer hazard (adjusted HR [HR_{adj}], 2.0; 95% CI, 1.1 to 3.6). This was entirely driven by an association with ER-positive disease (Fig 1A; ER-positive: HR_{adj}, 1.9; 95% CI, 1.1 to 3.5; ER-negative: HR_{adj}, 0.99; 95% CI, 0.14 to 7.0). The overall DBP association was modestly stronger among premenopausal women (Fig 1B). Lower levels of DBP exposure were not associated with breast cancer incidence. Estimates changed little between age-adjusted models and models additionally adjusted for menopausal status, other phthalate exposures, potentially confounding comedications, drug substance exposures, and CCI. Adjustment for parity had little effect on association estimates (data not shown).

TABLE 4. Associations Between Cumulative Phthalate Exposure and Incidence of ER-Positive and ER-Negative Invasive Breast Cancer, Denmark 2005 to 2015

Exposure	ER-Positive Breast Cancer		ER-Negative Breast Cancer	
	Adjusted HR*	95% CI	Adjusted HR*	95% CI
Dibutyl phthalate, mg				
Unexposed	1.0	Ref	1.0	Ref
1-249	0.92	0.73 to 1.2	1.1	0.66 to 1.8
250-999	0.65	0.35 to 1.2	1.5	0.55 to 3.9
1,000-9,999	0.71	0.43 to 1.2	0.26	0.04 to 1.8
≥ 10,000	1.9	1.1 to 3.5	0.99	0.14 to 7.0
Diethyl phthalate, mg				
Unexposed	1.0	Ref	1.0	Ref
1-9	1.1	0.93 to 1.2	1.2	0.88 to 1.5
10-99	0.98	0.92 to 1.1	1.2	1.0 to 1.4
≥ 100	0.93	0.84 to 1.0	1.0	0.80 to 1.3
Cellulose acetate phthalate, mg				
Unexposed	1.0	Ref	1.0	Ref
1-249	0.86	0.70 to 1.1	1.3	0.86 to 2.0
250-999	0.88	0.68 to 1.1	1.3	0.80 to 2.2
1,000-9,999	0.96	0.79 to 1.2	1.1	0.67 to 1.7
≥ 10,000	0.92	0.69 to 1.2	0.84	0.40 to 1.8
Hypromellose phthalate, mg				
Unexposed	1.0	Ref	1.0	Ref
1-249	1.0	0.82 to 1.2	0.59	0.29 to 1.2
250-999	0.96	0.87 to 1.1	1.2	0.95 to 1.5
1,000-9,999	0.87	0.78 to 0.98	1.0	0.78 to 1.3
≥ 10,000	0.83	0.65 to 1.0	0.46	0.21 to 1.0
Polyvinyl acetate phthalate				
Unexposed	1.0	Ref	1.0	Ref
Any exposure	0.91†	0.55 to 1.5	0.36†	0.05 to 2.6

Abbreviations: ER, estrogen receptor; HR, hazard ratio.
*Adjusted for age, menopausal status, and drug substance exposures.
†Adjusted for age.

DBP associations persisted in analyses restricted to exclusive users of lithium and mesalamine (Appendix Tables A3 and A4, online only). Among the exclusive lithium users, adjustment for lithium ion exposure did not attenuate DBP associations. Results were similar under the longer 2-year exposure lag and were somewhat attenuated (as expected) under no exposure lag (Appendix Fig A3, online only).

DISCUSSION

In accordance with our a priori hypothesis, we observed an approximately two-fold increase in breast cancer incidence associated with high cumulative exposure to DBP (≥ 10,000 mg). This association was observed for ER-positive but not for ER-negative disease and was stronger among premenopausal women. No other phthalate was associated with breast cancer incidence.

We did not observe a dose response in the hazard ratio with increasing levels of DBP exposure. It is possible that environmental DBP exposure in our reference group obscured any underlying dose-response relationship. It is also possible that DBP increases breast cancer risk only after exceeding a high DBP exposure threshold. Although the association with high DBP exposure was measured with good precision, we cannot rule out that it is an artifact of random error.

The association between DBP and ER-positive breast cancer is consistent with preclinical evidence concerning the estrogenic properties of phthalates and their metabolites. Studies show that DBP increases proliferation and viability in the ER-dependent MCF-7 breast cancer cell line,[20-22,25] although contradictory evidence also exists.[23] Preclinical studies implicating DBP in ER potentiation also
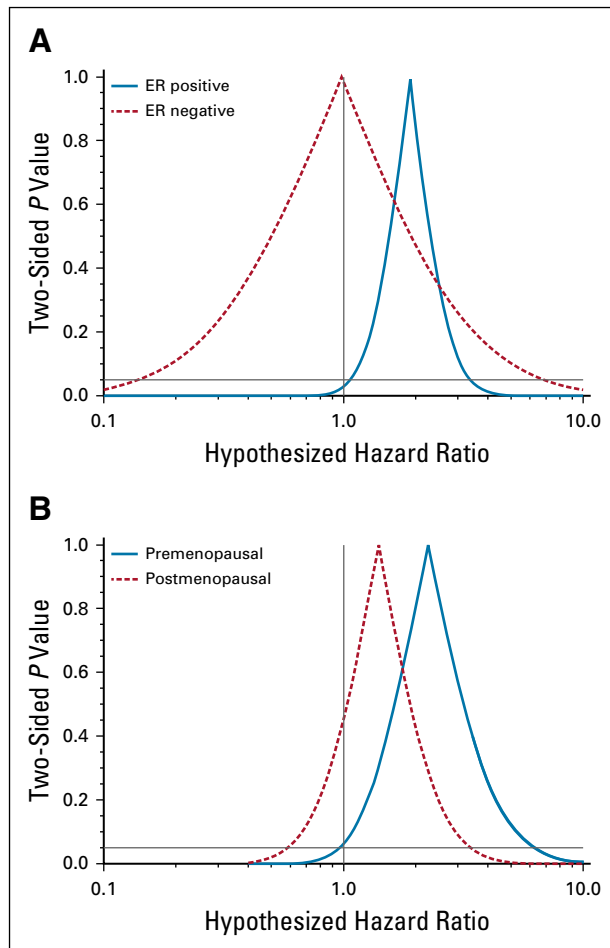
FIG 1. Confidence interval functions depicting adjusted associations between cumulative dibutyl phthalate exposure ($\geq$ 10,000 mg *v* no exposure) and incident breast cancer, (A) according to tumor estrogen receptor (ER) status, and (B) according to menopausal status.

showed similar effects for BBP and DEHP—two phthalates that we could not study because they were not used as excipients in any drugs marketed in Denmark.

Previous epidemiologic studies relied on urinary phthalate metabolites as a proxy for systemic exposure to parent compounds. Because of their rapid elimination, measurement of urinary metabolites captures only recent exposure. López-Carillo et al[30] found a potentially causal association of similar magnitude for a metabolite of DEP and potentially preventive associations for metabolites of BBP and DOP. Holmes et al[31] reported a potentially causal association for a metabolite of DEHP in a case-control study of Alaskan natives, and Morgan et al[32] found near-null associations between exposure to any phthalate metabolite and breast cancer in a cross-sectional US study. None of these studies found an association between DBP metabolites and incident breast cancer. Reeves et al[33] recently conducted the first prospective study of phthalate exposure and postmenopausal breast cancer incidence, using multiple measurements of urinary metabolites in 419 cases

and 838 matched controls. Although they concluded that there were no positive associations between phthalate exposures and breast cancer overall, they also reported a secondary analysis in which the highest quarter of DBP exposure was associated with 10-fold higher breast cancer odds compared with the lowest quarter (odds ratio, 9.96; 95% CI, 1.93 to 51), consistent with the DBP association pattern we observed.[33]

Primary strengths of our study are its prospective exposure information, large size, use of an essentially unselected nationwide source population, and high-validity data from the Danish population-based registries.[35,36,38,41,52] Using prescription fills to quantify phthalate exposure avoided costly biomarker assays. This not only permitted study of a large population but also facilitated the first epidemiologic study of breast cancer risk on the basis of time-varying and longitudinal measurement of phthalate exposure. In contrast, most earlier studies relied on relatively small numbers of patients and single, nonprospective exposure measurements.[30-32]

Our exposure measurement scheme has some notable tradeoffs, including misclassification of true phthalate exposure and potential confounding by active drug ingredients and the underlying medical indications for treatment. Some misclassification is expected for two reasons: first, we could not measure nonmedication sources of phthalate exposure (eg, occupational and environmental exposures); second, we cannot be certain that patients ingested all their filled prescriptions, as assumed in our exposure calculations. These concerns are allayed by the following considerations. First, on the basis of a prior exposure study, we expect environmental sources to make up a relatively small proportion of total exposure among users of phthalate-containing medications.[8,11] We would expect such misclassification to bias our association estimates toward the null, which would not explain the positive association between DBP and breast cancer risk. It is possible that such misclassification masked low-magnitude breast cancer associations with other phthalates. Second, misclassification as a result of incomplete adherence to prescription durations may have led to overestimation of cumulative exposure levels. However, a record in the DNPR means that a patient paid for and collected the medication, which implies intention to adhere. Furthermore, although absolute measures of cumulative ingestion may be overestimated, we expect that the rank-order of exposure levels would remain intact and serve well for comparison of disease risks between higher and lower exposure levels. Substantial confounding by active drug ingredients and medical indications is unlikely to have influenced our estimates. First, we expect that phthalate exposure in our cohort was randomly allocated among patients exposed to specific medications, as we are unaware of any influences on whether a given prescription was filled with a phthalate-containing product versus a phthalate-free product.

Unfortunately, polypharmacy in our study population prevented comparisons of phthalate exposure levels within strata of patients exposed to single medications, which would have preserved the natural randomization. However, the intermix of phthalate-containing and phthalate-free products across all medication types allowed us to adjust for drug substance exposures—and, simultaneously, for their underlying indications. Furthermore, none of the medications or treatment indications relevant to DBP exposure—bisacodyl (laxative for constipation), budesonide (corticosteroid for inflammatory bowel disease), diclofenac (nonsteroidal anti-inflammatory drug for arthritis and migraines), lithium (antipsychotic for bipolar disorder), mesalamine (aminosalicylate for inflammatory bowel disease), and multi-enzymes (for digestive aid)—has been associated with breast cancer incidence in clinical studies (Appendix Table A5).

We could not adjust for adiposity, which we expect to be higher among phthalate-exposed patients. However, adiposity is negatively associated with breast cancer risk in premenopausal women,[53] and the DBP association was particularly strong in that subgroup. Likewise, we could not adjust for complete reproductive history. However, adjustment for parity did not substantially affect association estimates—a result that is concordant with the expectation that parity would not be associated with phthalate exposures from medicines. Finally, our models of ER-positive and ER-negative disease could support adjustment only for age, menopausal status, and active drug ingredient exposures. Although this leaves the possibility of residual confounding due to other phthalate exposures, comedications, and comorbidity, these factors did not substantially affect association estimates from our main outcome models.

In summary, we observed an approximately two-fold increase in ER-positive breast cancer incidence among women who were highly exposed to DBP through medications, consistent with preclinical evidence and with our a priori hypothesis. No other type of phthalate exposure, including lower-level DBP exposure, was associated with breast cancer. BBP and DEHP have similar actions to DBP in vitro but could not be measured via medication use in Denmark. Future efforts should focus on these potentially important exposures in addition to replicating the DBP association. In the meantime, it may be prudent for women to consult with prescribers and pharmacists to determine the phthalate content of their medications and whether long-term treatment with DBP-formulated pharmaceuticals can be avoided.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Phthalate Exposure and Breast Cancer Incidence: A Danish Nationwide Cohort Study

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APPENDIX
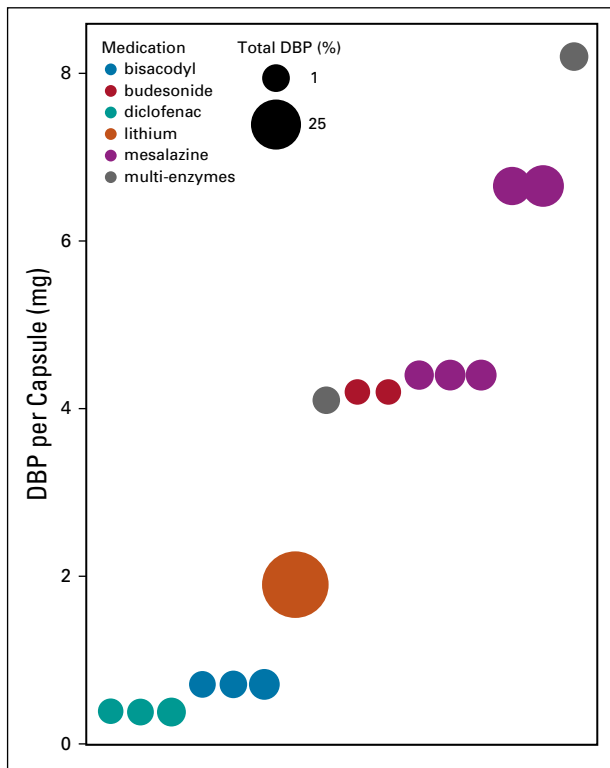
FIG A1. Contribution of specific drugs to the total mass of dibutyl phthalate (DBP) contained in prescriptions filled by the cohort. Lithium products make up 64% of the total DBP and mesalazine products make up 27% of the total.
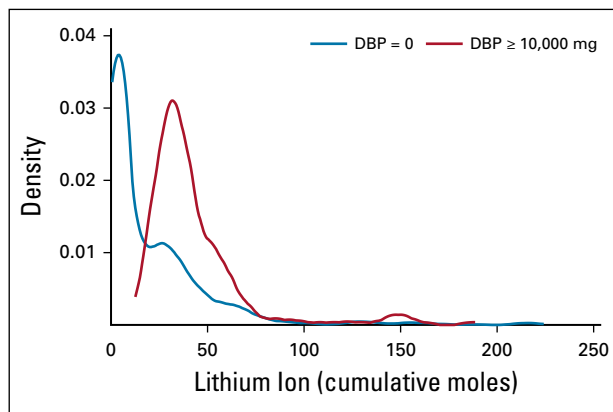
FIG A2. Distribution of cumulative moles of lithium ion in filled prescriptions for lithium drug products among exclusive users of lithium, according to dibutyl phthalate (DBP) exposure levels.
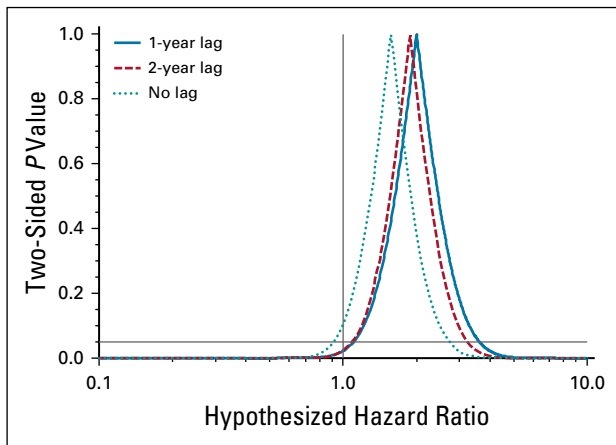
FIG A3. Adjusted associations between high dibutyl phthalate exposure (≥ 10,000 cumulative mg *v* unexposed) under lag periods of 2 years, 1 year (the main analysis), and 0 years (no lag).

TABLE A1. Characteristics of All Lithium Drug Products (ATC code: N05AN01) Prescribed to the Cohort

Product	Strength	DBP/Tablet (mg)	No. Exposed
Formulated with DBP	6.0 mmol Li⁺	1.9	2,171
Formulated without DBP	8.1 mmol Li⁺	0	2,072

Abbreviation: DBP, dibutyl phthalate.

TABLE A2. Characteristics of All Mesalamine Drug Products (ATC: A07EC02) Prescribed to the Cohort

Product Number	Strength (mg)	Tablets/Pack	DBP/Tablet (mg)	No. Exposed
1	800	60	6.7	423
2	800	180	6.7	523
3	400	100	4.4	210
4	400	300	4.4	158
5	250	100	4.4	63
6	400	100	0	35
7	800	60	0	94
8	800	180	0	262
9	1,000	28	0	286
10	1,000	30	0	318
11	1,000	30	0	598
12	1,000	28	0	726
13	1,000	60	0	150
14	1,200	60	0	94
15	400	100	0	113
16	400	100	0	37
17	400	300	0	88
18	500	300	0	801
19	400	300	0	116
20	1,000	28	0	557
21	1,000	28	0	117
22	1,500	70	0	4
23	400	100	0	197
24	400	300	0	121
25	1,000	28	0	101
26	1,000	14	0	249
27	500	100	0	256
28	500	300	0	272
29	500	100	0	375
30	800	60	0	1,435
31	800	180	0	2,068
32	400	100	0	306
33	400	300	0	230
34	500	300	0	367
35	1,000	28	0	1,011
36	1,000	700	0	817
37	500	100	0	764
38	1,000	150	0	70
39	1,000	30	0	877
40	500	100	0	22
41	500	300	0	537
42	1,200	60	0	178
43	800	180	0	802

TABLE A2. Characteristics of All Mesalamine Drug Products (ATC: A07EC02) Prescribed to the Cohort (continued)

Product Number	Strength (mg)	Tablets/Pack	DBP/Tablet (mg)	No. Exposed
44	400	300	0	49
45	400	300	0	2
46	500	60	0	1,342
47	1,000	700	0	156
48	1,000	700	0	755
49	1,000	700	0	18
50	1,000	700	0	29
51	1,000	14	0	61
52	250	100	0	61
53	500	60	0	1,879
54	2,000	60	0	66
55	500	100	0	293
56	500	300	0	641
57	3,000	50	0	12
58	400	100	0	7
59	3,000	50	0	1
60	800	60	0	233
61	1,200	60	0	125
62	1,000	30	0	468
63	2,000	60	0	83
64	800	60	0	15
65	1,000	60	0	291
66	1,200	60	0	140
67	1,000	14	0	15
68	1,000	700	0	40

Abbreviation: DBP, dibutyl phthalate

TABLE A3. Associations Between Dibutyl Phthalate Exposure and Breast Cancer Incidence, Restricted to Exclusive Users of Lithium or Mesalamine

Exposure	Cases	Person-Years	Age Adjusted		Multivariable*	
			HR	95% CI	HR	95% CI
Exclusive lithium users, mg						
Unexposed	37	17,272	1.0	Ref	1.0	Ref
1-249	≤ 5	900	0.50	0.07 to 3.7	0.50	0.07 to 3.7
250-999	≤ 5	1,367	0.33	0.05 to 2.5	0.34	0.05 to 2.5
1,000-9,999	8	3,243	1.2	0.53 to 2.6	1.2	0.53 to 2.6
≥ 10,000	≤ 5	599	3.9	1.3 to 12	4.0	1.3 to 12
Exclusive mesalamine users, mg						
Unexposed	87	38,052	1.0		1.0	
1-249	0	0	N/A	Ref	N/A	Ref
250-999	≤ 5	1,013	0.77	0.19 to 3.1	0.72	0.18 to 2.9
1,000-9,999	≤ 5	3,276	0.37	0.12 to 1.2	0.38	0.12 to 1.2
≥ 10,000	6	1,429	1.6	0.70 to 3.7	1.6	0.71 to 3.7

Abbreviations: HR, hazard ratio; N/A, not applicable.
*Adjusted for age, menopausal status, other phthalate exposures, use of potentially confounding comedications (cardiac glycosides, hormone therapy, aspirin, oral contraceptives, and statins), drug substance exposures, and Charlson comorbidity index.

TABLE A4. Baseline Characteristics of Patients Unexposed and Highly Exposed (≥ 10,000 cumulative mg) to DBP, Among Exclusive Users of Lithium or Mesalamine

Characteristic	Exclusive Lithium Users		Exclusive Mesalamine Users	
	No DBP Exposure (n = 1,410)	≥ 10,000 mg DBP (n = 185)	No DBP Exposure (n = 4,051)	≥ 10,000 mg DBP (n = 197)
Age, median (q1-q3)	50 (39-62)	46 (39-56)	48 (38-59)	48 (39-59)
Charlson comorbidity index				
0	1,245 (88)	170 (92)	3,617 (89)	175 (89)
1	120 (8.5)	13 (7.0)	334 (8.2)	15 (7.6)
2	30 (2.1)	0 (0)	75 (1.9)	5 (2.5)
3	15 (1.1)	2 (1.1)	25 (0.6)	2 (1.0)
Other phthalate exposures				
CAP	21 (1.5)	4 (2.2)	123 (3.0)	7 (3.6)
DEP	206 (15)	32 (17)	322 (8.0)	13 (6.6)
PVAP	67 (4.8)	13 (7.0)	9 (0.2)	0 (0)
HPMCP	178 (13)	20 (11)	270 (6.7)	16 (8.1)
Comedications				
Cardiac glycosides	6 (0.4)	1 (0.5)	31 (0.8)	3 (1.5)
Hormone therapy	183 (13)	15 (8.1)	449 (11)	30 (15)
Aspirin	69 (4.9)	4 (2.2)	225 (5.6)	14 (7.1)
Statins	54 (3.8)	2 (1.1)	189 (4.7)	12 (6.1)
Oral contraceptives	140 (9.9)	29 (16)	621 (15)	34 (17)

NOTE. Data given as No. (%) unless otherwise noted.
Abbreviations: CAP, cellulose acetate phthalate; DBP, dibutyl phthalate; DEP, diethyl phthalate; HPMCP, hydroxypropyl methylcellulose phthalate; PVAP, polyvinyl acetate phthalate.

TABLE A5. Characteristics of the Prescription Drug Products That Contributed to Dibutyl Phthalate Exposure in the Cohort

Drug	ATC Code	Strength	DBP/Tablet (mg)	No. Exposed
Bisacodyl	A06AB02	5 mg	0.71	783
		5 mg	0.71	818
		5 mg	0.71	397
Budesonide	A07EA06	3 mg	4.2	48
		3 mg	4.2	57
Diclofenac	M01AB05	75 mg	0.38	2,588
		75 mg	0.38	573
		75 mg	0.38	547
Lithium	N05AN01	6.0 mmol Li$^+$	1.9	2,171
Mesalamine	A07EC02	800 mg	6.7	423
		800 mg	6.7	523
		400 mg	4.4	210
		400 mg	4.4	158
		250 mg	4.4	63
Multi-enzymes	A09AA02	Lipase, 25 units	8.2	43
		Lipase, 10 units	4.1	76

Abbreviations: ATC, Anatomic Therapeutic Chemical code; DBP, dibutyl phthalate.