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Community water service and incidence of respiratory, skin, and gastrointestinal infections in rural Alaska, 2013–2015

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ABSTRACT

Background: Communities in rural Alaska have access to multiple types of water service (piped, vehicle-hauled, and self-hauled) and experience varying levels of water service coverage. We assessed the incidence rate of inpatient and outpatient infectious disease visits among communities with different water service types and coverage levels.

Methods: We classified ICD-9 codes for inpatient and outpatient visits to the Yukon-Kuskokwim Health Corporation facilities between 2013 and 2015 into six infectious disease categories. Using Poisson models, we compared the incidence of visits in each category across communities with differing water service coverage levels as defined by water service billing data for the same years. Using census data, we adjusted for community median household income, median age, crowding, and health aide staffing.

Results: We included 48 communities in this analysis. After adjusting for possible confounders, each 10% increase in piped water coverage was associated with a 4% lower incidence of pneumonia/influenza visits (adjusted incidence rate ratio [IRR] 0.96, 95% CI 0.93–0.98), a 2% lower incidence of other respiratory infection visits (adjusted IRR 0.98, 95% CI 0.97–0.99), an 8% lower incidence of methicillin-resistant *Staphylococcus* visits (adjusted IRR 0.92, 95% CI 0.87–0.97), and a 4% lower incidence of other skin infections visits (adjusted IRR 0.96, 95% CI 0.95–0.98). Each 10% increase in vehicle-hauled water coverage was associated with a 2% lower incidence of respiratory infection visits (adjusted IRR 0.98, 95% CI 0.97–0.996) and a 3% lower incidence of skin infection visits (adjusted IRR 0.97, 95% CI 0.95–0.99), also after adjustment.

Conclusions: Higher levels of water service coverage were associated with lower incidence rates of visits for several infectious disease categories. These associations were more pronounced for communities with piped water service compared to vehicle-hauled water service.

1. Introduction

Globally, access to clean water and sanitation is associated with lower mortality and disease (Fink et al., 2011; Wolf et al., 2014). However, even in high income countries, adequate water and sanitation access are not always guaranteed. Within the United States, Alaska has the lowest proportion of homes with complete plumbing, defined as hot and cold piped water, a flush toilet and a bathtub or shower (United States Census Bureau, 2013). The lack of in-home piped water in Alaska has been associated with increased infectious disease morbidity including respiratory hospitalizations in children, skin infections, gastrointestinal infections, and invasive pneumococcal disease (Thomas et al., 2016; Hennessy et al., 2008; Bulkow et al., 2012; Wenger et al., 2010).

The Yukon-Kuskokwim (YK) Delta is a remote rural area in western Alaska where three systems of water provision are employed. Some communities have piped water service, where water is distributed from a centralized source or well directly to homes. Some communities have

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a small vehicle haul system, where small (120 gallon) water and sewage holding tanks are installed at the home and a community vehicle (such as an all-terrain vehicle or snow mobile) brings potable water to the home and removes sewage from these tanks. Finally, families in communities without in-home piped water or a small vehicle haul system must haul water from a community clean watering point and haul waste to a sewage disposal site themselves. Even if the water source is high quality, stored water in communities such as these has been found to become easily contaminated if left uncovered or when hands or utensils are dipped into a storage container (Overbo et al., 2016).

Among communities that have piped or vehicle-hauled water service systems, water service coverage in the YK Delta is variable both across and within communities. Communities often face maintenance challenges due to high costs, harsh weather conditions, challenging soil, electrical power interruptions, and lack of skilled operators. Additionally, failure to pay water and sewer bills leads to discontinuation of service for some households, leading to lower water service coverage in the community.

The infectious disease risk associated with not having in-home piped water in Alaska was last quantified between 2000 and 2004. This analysis serves as an update to the previous publication and an evaluation of the rates of infectious associated with each of the three types of water service, which has not been previously evaluated.

2. Methods

2.1. Study population

This study was conducted among communities in the YK Delta in western Alaska. The YK Delta covers 50,000 square miles and consists primarily of tundra. Approximately 25,000 people live in the YK Delta, and 89% of residents are Alaska Native people (United States Census Bureau, 2010).

This protocol was approved by the Alaska Area institutional review board under protocol number 929311.

2.2. Data sources

Disease outcome data were extracted from the YK Delta regional health organization electronic health record system, Records and Verification Electronic Network (RAVEN). We requested a de-identified dataset including visit date, residence location, and diagnosis code for all inpatient and outpatient visits using specified International Classification of Diseases, 9th Revision (ICD-9) codes to capture respiratory, skin, and gastrointestinal infections of interest for the years 2013, 2014, and 2015. The ICD-9 codes requested are included in Supplementary Table 1.

The YK Health Corporation Office of Environmental Health and Engineering (OEHE) provided water service coverage data for the region, consisting of the number of households in each community and number of households billed for piped or hauled water service for the years 2013, 2014, and 2015. The YK OEHE also provided yearly data regarding health aide staffing for each community. This staffing was parameterized as the percent of a full time health aide employed in the community over the course of one year. For example, if one health aide was employed in the community for 6 months and then no health aides were employed, the value would be 0.5.

Other community-level data, such as median income, gender distribution, and crowding were extracted from the 2016 American Community Survey of the United States Census.

2.3. Variable definitions

Disease categorizations and water service types were defined to align with a previous paper assessing this question (Hennessy et al., 2008). The disease categories included diarrheal disease, pneumonia/influenza, Respiratory Syncytial Virus (RSV), other infectious respiratory diseases, methicillin-resistant *Staphylococcus aureus*, and other infectious skin diseases.

Communities in the YK Delta have varying levels of coverage by piped water service and vehicle-hauled water service (hereafter "hauled" service), and some communities have both. We created categorical variables to reflect the community's primary water source as follows: low/no service (less than 10% of households were billed for either piped water or haul service), primarily haul service (over 10% of households were billed for hauled service, but less than 50% were billed for piped service), moderate piped service (over 10% but less than 80% of households were billed for piped service [and less than 10% hauled service]), and high piped service (over 80% of households were billed for piped service [and less than 10% hauled service]). These categories were chosen to align with a previous paper analyzing this question (Hennessy et al., 2008). For models (as described below), coverage of each service was used as a continuous variable reflecting the percentage of households that were billed for service each year.

Crowding was defined as the percent of households with greater than 1.5 occupants per room, according to the US Census (United States Census Bureau, 2010). Payment to community health aides for each community was used to represent the level of health aide staffing. This value could be less than one if the community did not have a full-time health aide for the entire year.

2.4. Statistical methods

Communities were described by population size, median household income, median age, percent female, percent crowded housing units, and health aide staffing across primary water service categories. We calculated incidence rates for each disease category by year and water service category using the number of visits and the total population of each community. Trends in crude incidence rates were evaluated using Poisson regression.

We analyzed the primary question of the community-level association between water service coverage and infectious disease incidence rates using mixed-effects Poisson models, producing incidence rate ratios (IRR). Water service coverage was included as a time-varying predictor over the course of the study period.

In order to create clean comparisons between communities with varying levels of each service type, we conducted the models in three groups of communities. The first group excluded any communities with hauled water service to allow comparison of incidence rates across varying proportions of piped service (Comparison A). The second group excluded any communities with piped water service to allow comparison of incidence rates across varying proportions of hauled service (Comparison B). To compare piped water service communities to hauled water service communities, we used a group that only included communities with at least 10% coverage of either piped or hauled water service (Comparison C). For this comparison, we evaluated incidence rates using a binary predictor comparing communities with hauled water service to communities with piped water service, while controlling for coverage percentage of either service. After evaluating unadjusted associations in each model, we controlled for community median income, median age, crowding percentage, and level of health aide staffing. We chose these potential confounders by creating a directed acyclic graph (Supplemental Fig. 1).

3. Results

This analysis included 48 communities, with a total population of 19,757 people, in the YK Delta. The largest number of people (9499 people, 48%) lived in communities with between 10% and 80% coverage of piped water. Roughly even proportions of people lived in communities with no service or less than 10% coverage of either service (3411, 17%), primarily hauled service (3656, 19%), or over 80% piped

Table 1
Characteristics of included communities, YK Delta, Alaska, 2013–2015.

	No or < 10% service	≥10% haul service[a]	≥10% but < 80% piped service	≥80% piped service
Number of communities	12	10	19	7
Total Population	3411	3656	9599	3091
Median household income, mean(SD), USD	35719 (10872)	37648(6247)	38054(6455)	40636(11616)
Median age in years, mean(SD)	28(5)	28(7)	25(4)	26(3)
Percent female, mean(SD)	44(6)	50(4)	47(4)	49(4)
Percent crowded housing units[b], mean(SD)	23(18)	22(15)	28(12)	22(13)
Mean health aide staffing	1.9 (1.1)	2.3 (0.75)	2.6 (1.9)	2.2 (1.1)

[a] And less than 50% piped service.
[b] Defined as percent of housing units with 1.51 or more people per room.

service ([3091, 16%] Table 1). Across communities, the median service coverage of either type was 61%.

Between 2013 and 2015, 55,496 other respiratory visits, 17,166 other skin infection visits, 7,357 pneumonia/influenza visits, 2,432 diarrheal disease visits, 329 methicillin-resistant *Staphylococcus aureus* (MRSA) infection visits, and 78 RSV visits were recorded through YKHC RAVEN electronic medical records. Accordingly, rates of outpatient visits during this time period were highest for respiratory infections, including pneumonia/influenza infections, and skin infections. The crude rates of pneumonia/influenza, RSV, other respiratory infections, MRSA and skin infections show a trend toward higher rates among communities with low service and lower rates among communities with high service (Table 2). Inpatient visit rates were smaller than outpatient rates and did not show clear trends across service categories (Table 3).

Between 2013 and 2015, 29 communities had at least some piped water service for at least one year. Piped water service coverage ranged from 9% to 100% of households, with a median coverage of 74% of households in these communities. Among communities that had low or no hauled water service (Comparison A; Table 4), for each 10% increase in piped water service coverage, communities had a 7% lower incidence of pneumonia/influenza visits (unadjusted IRR 0.93, 95% CI 0.90–0.95), a 9% lower incidence of MRSA visits (unadjusted IRR 0.91, 95% CI 0.86–0.96), and a 4% lower incidence of other skin infection visits (unadjusted IRR 0.96, 95% CI 0.94–0.97). After adjusting for median income, median age, crowding, and health aide staffing, each 10% increase in piped water coverage was associated with a 4% lower incidence of pneumonia/influenza visits (adjusted IRR 0.96, 95% CI 0.93–0.98), a 2% lower incidence of other respiratory infection visits (adjusted IRR 0.98, 95% CI 0.97–0.99), an 8% lower incidence of MRSA visits (adjusted IRR 0.92, 95% CI 0.87–0.97), and a 4% lower incidence of other skin infection visits (adjusted IRR 0.96, 95% CI 0.95–0.98).

Between 2013 and 2015, 13 communities had hauled water service for at least one year. Hauled water service coverage ranged from 11% to 98% of households, with a median coverage of 50% of households. Among communities that had low or no piped service (Comparison B; Table 5), for each 10% increase in hauled water service coverage, communities had a 2% lower incidence of respiratory infection visits (unadjusted IRR 0.0.98, 95% CI 0.97–0.996) and a 4% lower incidence of skin infection visits (unadjusted IRR 0.96, 95% CI 0.94–0.99). After adjusting for median income, median age, crowding, and health aide staffing, each 10% increase in hauled water coverage was associated with a 2% lower incidence of respiratory infection visits (adjusted IRR 0.98, 95% CI 0.97–0.996) and a 3% lower incidence of skin infection visits (adjusted IRR 0.97, 95% CI 0.95–0.99).

Table 2
Infectious disease outpatient clinic visit rates per 10,000 people by water service type and year, YK Delta, Alaska, 2013–2015.

	Service category							
	None or < 10%		≥10% haul[a]		≥10% but < 80% piped		≥80% piped	
	Visits	Rate	Visits	Rate	Visits	Rate	Visits	Rate
Infectious Diarrhea								
2013	181	288.3	57	212.0	228	297.9	90	287.0
2014	209	461.9	100	331.0	417	434.6	103	393.9
2015	129	378.2	109	364.8	395	405.8	141	389.1
Pneumonia/influenza								
2013	887	1412.6	346	1286.7	1062	1387.7	299	953.4
2014	591	1306.1	545	1804.0	1382	1440.2	233	891.0
2015	318	932.3	289	967.2	834	856.8	225	620.9
RSV								
2013	4	6.4	1	3.7	6	7.8	1	3.2
2014	10	22.1	3	9.9	8	8.3	1	3.8
2015	5	14.7	1	3.3	11	11.3	1	2.8
Other respiratory infection								
2013	5000	7963.1	2359	8772.8	5931	7749.9	1993	6355.2
2014	4588	10139.2	3523	11661.7	8801	9171.5	2132	8153.0
2015	3849	11284.1	3944	13199.5	9808	10076.0	3414	9420.5
MRSA								
2013	11	17.5	5	18.6	10	13.1	4	12.8
2014	21	46.4	6	19.9	17	17.7	2	7.6
2015	1	2.9	6	20.1	14	14.4	2	5.5
Other skin infection								
2013	2775	4419.5	805	2993.7	1968	2571.5	474	1511.5
2014	1790	3955.8	853	2823.6	2620	2730.3	412	1575.5
2015	1112	3260.0	930	3112.4	2461	2528.3	648	1788.1

[a] And less than 50% piped service.

Table 3
Infectious disease inpatient clinic visit rates per 10,000 people by water service type and year, YK Delta, Alaska, 2013–2015.

	Service category							
	None or < 10%		≥10% haul[a]		≥10% but < 80% piped		≥80% piped	
	Visits	Rate	Visits	Rate	Visits	Rate	Visits	Rate
Infectious Diarrhea								
2013	34	54.1	10	37.2	41	53.6	10	31.9
2014	21	46.4	21	69.5	55	57.3	11	42.1
2015	15	44.0	12	40.2	34	34.9	9	24.8
Pneumonia/influenza								
2013	44	70.1	17	63.2	42	54.9	11	35.1
2014	41	90.6	31	102.6	68	70.9	14	53.5
2015	15	44.0	12	40.2	41	42.1	10	27.6
RSV								
2013	1	1.6	1	3.7	2	2.6	0	0.0
2014	4	8.8	1	3.3	9	9.4	1	3.8
2015	0	0.0	1	3.3	5	5.1	1	2.8
Other respiratory infection								
2013	10	15.9	6	22.3	15	19.6	11	35.1
2014	19	42.0	7	23.2	29	30.2	3	11.5
2015	9	26.4	7	23.4	29	29.8	9	24.8
MRSA								
2013	27	43.0	11	40.9	24	31.4	3	9.6
2014	32	70.7	26	86.1	46	47.9	6	22.9
2015	13	38.1	13	43.5	26	26.7	3	8.3
Other skin infection								
2013	38	60.5	14	52.1	26	34.0	4	12.8
2014	43	95.0	28	92.7	57	59.4	5	19.1
2015	24	70.4	16	53.5	56	57.5	7	19.3

[a] And less than 50% piped service.

Among communities that had at least 10% coverage of either service, communities that had piped water service had a 37% lower incidence of pneumonia/influenza visits (unadjusted IRR 0.55, 95% CI 0.41–0.74, adjusted IRR 0.63, 95% CI 0.45–0.88) compared to communities with hauled water service (Comparison C; Table 6). However, in other disease categories, communities with piped water service did not have significantly different visit incidence rates compared to communities with hauled water service.

4. Discussion

We evaluated the association between water service type and the incidence of infectious disease visits in the YK Delta. We found that higher coverage of piped and hauled water service was associated with lower incidence of several categories of infectious disease inpatient and outpatient visits, even after controlling for other differences between communities. Higher piped water service coverage was associated with lower pneumonia/influenza visit rates, other respiratory infection visit rates, MRSA visit rates, and other skin infection visit rates. Higher hauled water service coverage was associated with lower respiratory infection rates and skin visit rates. These results have broad relevance for other Arctic settings, for example northern Canada or Greenland, which often have similar water provision methods (Hendriksen and Hoffmann, 2018; Bressler and Hennessy, 2018; Daley et al., 2018).

This analysis serves as an update to the evaluation of water service and infectious disease visits conducted in between 1999 and 2004 in Alaska. In the previous study, communities were categorized using water service as a binary indicator (served or unserved) and by categories of coverage percentage for any service. Communities with piped water or vehicle-hauled water service were both categorized as having water service, while those requiring self-hauling were categorized as not having water service (Hennessy et al., 2008). The previous analysis identified that pneumonia/influenza visit rates, skin infection visit rates, and MRSA infection visit rates were lower in areas with water

Table 4
Community-level relative risk of infectious disease outpatient or hospitalization visits by **piped water service proportion**, among communities with low or no haul service (33 communities, 89 observations) YK Delta, Alaska, 2013–2015[a].

	Relative Risk (95% CI), unadjusted	Relative Risk (95% CI), adjusted
Infectious Diarrhea		
Proportion with piped water[b]	1.01 (0.97, 1.04)	1.01 (0.98, 1.04)
Median income, USD[c]		1.00 (0.99, 1.02)
Median age, years		1.02 (0.98, 1.06)
Crowding		1.08 (0.79, 1.47)
Health Aide[d]		**1.12 (1.07, 1.18)**
Pneumonia/influenza		
Proportion with piped water[b]	**0.93 (0.90, 0.95)**	**0.96 (0.93, 0.98)**
Median income, USD[c]		1.02 (0.99, 1.05)
Median age, years		**0.92 (0.86, 0.99)**
Crowding		1.24 (0.71, 2.17)
Health Aide		**0.91 (0.88, 0.94)**
RSV		
Proportion with piped water[b]	0.97 (0.88, 1.06)	0.94 (0.86, 1.02)
Median income, USD[c]		1.01 (0.97, 1.05)
Median age, years		0.99 (0.88, 1.12)
Crowding		1.01 (0.46, 2.19)
Health Aide		**1.51 (1.31, 1.74)**
Other respiratory infections		
Proportion with piped water[b]	1.00 (0.99, 1.01)	**0.98 (0.97, 0.99)**
Median income, USD[c]		**1.02 (1.01, 1.04)**
Median age, years		**0.95 (0.92, 0.99)**
Crowding		1.17 (0.85, 1.60)
Health Aide		**1.10 (1.08, 1.11)**
MRSA		
Proportion with piped water[b]	**0.91 (0.86, 0.96)**	**0.92 (0.87, 0.97)**
Median income, USD[c]		1.01 (0.99, 1.04)
Median age, years		1.01 (0.93, 1.10)
Crowding		**1.77 (1.03, 3.05)**
Health Aide		1.06 (0.94, 1.20)
Other skin infections		
Proportion with piped water[b]	**0.96 (0.94, 0.97)**	**0.96 (0.95, 0.98)**
Median income, USD[c]		**1.03 (1.01, 1.05)**
Median age, years		0.96 (0.91, 1.01)
Crowding		1.33 (0.97, 1.01)
Health Aide		0.99 (0.97, 1.02)

[a] Comparison A, as described in the text.
[b] For a 10% increase in coverage.
[c] For a 1000 USD increase in median income.
[d] Units: One full time health aide employed in the community.

service (Hennessy et al., 2008). In the current study, we identified the same associations between visit rates and increasing levels of piped water service, with the addition of lower rates for other respiratory infection visits as well. These are considered “water-washed” diseases: even though they are not directly transmitted through water, their transmission is linked to water availability and its relationship with hygiene (Hennessy et al., 2008). Diarrheal disease visit rates were not associated with water service in either study (Hennessy et al., 2008). However, in a prospective study of four villages that received water services, respiratory, skin, and diarrheal disease rates all significantly declined after receipt of services (Thomas et al., 2016). The lack of association in the current study could be because the water quality is high, even though quantity is low, the cross-sectional nature of the study, or that care-seeking behaviors for diarrheal disease is low, leading to underassessment (Hansdotter et al., 2015). Future studies

Table 5
Community-level relative risk of infectious disease outpatient or hospitalization visits by **hauled water service proportion**, among communities with low or no piped service (19 communities and 52 observations) YK Delta, Alaska, 2013–2015.

	Relative Risk (95% CI), unadjusted	Relative Risk (95% CI), adjusted
Infectious Diarrhea		
Proportion with hauled water[a]	0.96 (0.92, 1.02)	0.98 (0.94, 1.02)
Median income, USD[b]		1.00 (0.97, 1.03)
Median age, years		1.05 (1.01, 1.09)
Crowding		1.14 (0.76, 1.70)
Health Aide[c]		**1.16 (1.04, 1.29)**
Pneumonia/influenza		
Proportion with hauled water[a]	1.02 (0.98, 1.05)	1.02 (0.99, 1.06)
Median income, USD[b]		1.03 (0.99, 1.08)
Median age, years		0.95 (0.89, 1.01)
Crowding		0.90 (0.48, 1.67)
Health Aide		**0.91 (0.86, 0.97)**
RSV		
Proportion with hauled water[a]	0.88 (0.75, 1.03)	0.87 (0.73, 1.04)
Median income, USD[b]		1.04 (0.93, 1.16)
Median age, years		0.94 (0.74, 1.20)
Crowding		0.48 (0.10, 2.34)
Health Aide		1.49 (0.90, 2.49)
Other respiratory infections		
Proportion with hauled water[a]	**0.98 (0.97, 0.996)**	**0.98 (0.97, 0.996)**
Median income, USD[b]		1.01 (0.97, 1.05)
Median age, years		**0.97 (0.92, 1.02)**
Crowding		0.98 (0.56, 1.71)
Health Aide		**1.23 (1.20, 1.25)**
MRSA		
Proportion with hauled water[a]	1.03 (0.96, 1.10)	1.06 (0.99, 1.13)
Median income, USD[b]		0.98 (0.95, 1.02)
Median age, years		**0.90 (0.82, 0.98)**
Crowding		1.47 (0.85, 2.53)
Health Aide		0.98 (0.80, 1.19)
Other skin infections		
Proportion with hauled water[a]	**0.96 (0.94, 0.99)**	**0.97 (0.95, 0.99)**
Median income, USD[b]		1.02 (0.99, 1.06)
Median age, years		**0.95 (0.90, 0.996)**
Crowding		0.58 (0.95, 2.63)
Health Aide		0.96 (0.93, 1.01)

*Comparison B, as described in the text.
[a] For a 10% increase in coverage.
[b] For a 1000 USD increase in median income.
[c] Units: One full time health aide employed in the community.

Table 6
Community-level relative risk of infectious disease outpatient or hospitalization visits **comparing piped water service to hauled water service**, among communities with any water service (31 communities and 85 observations) YK Delta, Alaska, 2013–2015.

	Relative Risk (95% CI), unadjusted	Relative Risk (95% CI), adjusted
Infectious Diarrhea		
Piped water community[a]	0.81 (0.55, 1.19)	0.98 (0.73, 1.33)
Coverage percentage		**0.91 (0.86, 0.96)**
Median income, USD[b]		0.99 (0.97, 1.01)
Median age, years		**1.12 (1.08, 1.16)**
Crowding		1.11 (0.79, 1.56)
Health Aide		**1.20 (1.11, 1.29)**
Pneumonia/influenza		
Piped water community[a]	**0.55 (0.41, 0.74)**	**0.63 (0.45, 0.88)**
Coverage percentage		0.97 (0.91, 1.02)
Median income, USD[b]		1.01 (0.98, 1.05)
Median age, years		1.02 (0.96, 1.05)
Crowding		1.49 (0.82, 2.71)
Health Aide[c]		0.97 (0.92, 1.02)
RSV		
Piped water community[a]	0.75 (0.34, 1.67)	0.72 (0.32, 1.62)
Coverage percentage		0.93 (0.81, 1.08)
Median income, USD[b]		1.00 (0.94, 1.05)
Median age, years		1.03 (0.90, 1.18)
Crowding		1.16 (0.46, 2.95)
Health Aide[c]		**1.46 (1.21, 1.76)**
Other respiratory infections		
Piped water community[a]	**1.19 (1.07, 1.32)**	1.15 (1.00, 1.31)
Coverage percentage		0.98 (0.95, 1.00)
Median income, USD[b]		1.00 (0.98, 1.02)
Median age, years		1.02 (0.98, 1.07)
Crowding		1.08 (0.75, 1.56)
Health Aide[c]		**1.16 (1.14, 1.19)**
MRSA		
Piped water community[a]	1.76 (0.31, 9.86)	0.89 (0.14, 5.78)
Coverage percentage		1.30 (0.88, 1.91)
Median income, USD[b]		1.04 (0.89, 1.20)
Median age, years		0.98 (0.62, 1.55)
Crowding		2.03 (0.88, 1.97)
Health Aide[c]		
Other skin infections		
Piped water community[a]	0.84 (0.68, 1.04)	0.93 (0.76, 1.14)
Coverage percentage		0.97 (0.93, 1.01)
Median income, USD[b]		1.01 (0.99, 1.02)
Median age, years		1.00 (0.97, 1.04)
Crowding		1.06 (0.80, 1.40)
Health Aide[c]		1.02 (0.97, 1.06)

*Comparison C, as described in the text.
[a] Being in a community with more than 10% piped water coverage.
[b] For a 1000 USD increase in median income.
[c] Units: One full time health aide employed in the community.

incorporating water quality assessments could clarify the reasons for this lack of association.

We found that higher coverage of hauled water service was associated with lower rates of MRSA and respiratory infection visits. Hauled water systems allow the household to have a closed system including toilet, a shower, and one or two sinks (Eichelberger, 2010). Although this system can minimize contamination of the water, it does not substantially improve the amount of water available for use in a household. An analysis of a community using hauled water showed that closed haul systems provided approximately 4 gallons of water per capita per day (GPCD) (Altiok, 2008). In self-haul communities, the average water usage has been estimated to be 2 GPCD (Thomas et al., 2016). By contrast, the average water usage in the United States, where the majority of residents use piped water, has been estimated at 69 GPCD (DeOreo, 1999). Although hauled communities are considered to be "underserved" by these standards, (Hickel et al., 2018) our analysis shows a slight benefit from the system compared to communities with no water provision (Comparison B). However, we found that pneumonia/influenza visit rates were much lower among piped water communities compared to hauled water communities, even after accounting for differences in service coverage (Comparison C). This suggests that the amount of water provided through vehicle haul systems may be insufficient to provide the full benefits that piped water affords.

The rates of disease were also associated with community median age (pneumonia/influenza, other respiratory infections), crowding (MRSA), and health aide staffing (diarrheal disease, pneumonia/influenza, other respiratory infections). The included communities have populations with high numbers of children, which could explain why

higher median age was associated with lower respiratory infection rates. In the YK Delta, MRSA has previously been associated with household member nasal carriage, transmission of which could be associated with crowding (Stevens et al., 2010). Finally, communities with health aides have greater access to care than those without health aides, leading these communities to have higher rates of healthcare visits for infectious diseases.

4.1. Limitations

This analysis is limited by a few primary factors. First, the number of communities was small, limiting the power of the analysis. Although we used 3 years of data for each community, the additional power gained through the multiple time points may not have been sufficient to assess modest associations. Additionally, some communities had both piped and hauled water service, although the number of these communities was small (two to three per year). In future studies, this issue could be resolved by evaluating household-level water access and disease outcomes. Although we controlled for community differences in income, crowding, and health aide staffing, there may be additional unmeasured factors that could influence the differences in disease rates. Finally, because we were evaluating rates and community-level service type and coverage, this analysis could be subject to ecological fallacy, wherein the conclusions drawn from the aggregate data do not reflect individual level associations. However, the use of a continuous exposure variable makes this potential for bias less likely.

5. Conclusions

In these communities in the YK Delta, higher water service coverage was associated with lower incidence rates of visits for several infectious disease categories. According to the relative risk ratios we calculated, compared to a community with no piped water service, a community with 100% coverage of piped water would have 90% fewer visits for pneumonia/influenza, 20% fewer visits for other respiratory infections, 80% fewer visits for MRSA, and 40% fewer visits for other skin infections. The effects observed here were more evident among communities with piped water coverage compared to vehicle-hauled water coverage. Compared to a community with no hauled water service, a community with 100% coverage of hauled water would have 20% fewer visits for respiratory infections and 30% fewer visits for MRSA. Improving water service coverage in rural Alaska, specifically of piped water, could meaningfully decrease the burden of infectious disease in these communities.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry. Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

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Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2020.113475.

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Review

How to assess respiratory sensitization of low molecular weight chemicals?

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ABSTRACT

There are no validated and regulatory accepted (animal) models to test for respiratory sensitization of low molecular weight (LMW) chemicals. Since several decades such chemicals are classified as respiratory sensitizers almost exclusively based on observations in workers. However, both respiratory allergens (in which process the immune system is involved) as well as asthmagens (no involvement of the immune system) may induce the same type of respiratory symptoms. Correct classification is very important from a health's perspective point of view. On the other hand, over-classification is not preferable in view of high costs to overdue workplace engineering controls or the chemical ultimately being banned due to Authorities' decisions. It would therefore be very beneficial if respiratory sensitizers can be correctly identified and distinguished from skin sensitizers and non-sensitizers/respiratory irritants. The purpose of this paper is to consider whether LMW chemicals can be correctly identified based on the currently available screening methods in workers, and/or via *in silico*, *in vitro* and/or *in vivo* testing. Collectively, based on the available information further effort is still needed to be able to correctly identify respiratory sensitizers and to distinguish these from skin sensitizers and irritants, not at least because of the far-reaching consequences once a chemical is classified as a respiratory sensitizer.

1. Introduction

Under the terms of the Globally Harmonized System (GHS) of Classification, an LMW chemical currently classified as a respiratory sensitizer (H334) has as Hazard Statement: '*may cause allergy or asthma symptoms or breathing difficulties if inhaled*' (GHS, 2011). The guidance provided by the European Chemicals Agency (ECHA) for the implementation of REACH (ECHA, 2016) states that a respiratory sensitizer is defined '*as an agent that will lead to hypersensitivity of the airways following inhalation exposure of that agent.*' The guidance also indicates that: '*Respiratory sensitization (or hypersensitivity) is a term that is used to describe asthma and other related respiratory conditions (rhinitis, extrinsic allergic alveolitis), irrespective of the mechanism (immunological or non-immunological) by which they are caused.*'

Thus, this definition includes both true respiratory allergens which, by definition, induce effects via immunological mechanisms, and non-allergenic asthmagens in which case adverse effects are caused by non-immunological mechanisms (Kimber et al., 2001; North et al., 2016).

True chemical respiratory allergy is characterised by immunological priming that results in allergic sensitization of the respiratory tract. If the sensitized subject is exposed subsequently by inhalation to the same chemical then an accelerated and aggressive secondary immune response will be provoked that elicits a respiratory reaction recognised clinically as respiratory allergy, and in a workplace setting described as occupational asthma. Reactions may become more severe with repeated exposure as the level of sensitization increases (Kimber et al., 2011).

It may be argued that because both types of chemicals, viz. respiratory allergens and asthmagens, may cause the same type of respiratory reactions there would be no harm to have these classified in the same category to protect workers or the general population. Or in other words, it would be better to over-classify rather than under-classify. However, classification for respiratory sensitization does not really take potency into account (see further) and asthmagens generally cause problems only at high and irritating concentrations. Thus, a chemical being classified as respiratory sensitizer but being an asthmagen will not only immediately require the implementation of highly protective measures at high costs; it will also be considered a Substance of Very High Concern (SVHC) under EU REACH with far going consequences such as restriction or authorization with the final aim of having the chemical banned.

In this paper, the scope and toxicology of chemical respiratory allergy will be briefly reviewed as well as the approaches currently available for the identification of LMW chemicals that have the ability to cause sensitization of the respiratory tract.

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2. Chemical respiratory allergy

Chemical respiratory allergy induced by Low Molecular Weight (LMW) chemicals is associated with a variety of occupational health and toxicological challenges, and these have been reviewed elsewhere (Holsapple et al., 2006; Kimber et al., 2007, 2014a; 2014b; Boverhof et al., 2008; Isola et al., 2008; Basketter and Kimber, 2011; Dotson et al., 2015; Cochrane et al., 2015; North et al., 2016; Chary et al., 2018).

LMW chemicals (those less than 500 Da) are not of sufficient size to engage effectively with the immune system in order to provoke an immune response; chemicals must be inherently electrophilic or must be transformed *in vivo* to an electrophilic species. Next, they should form a stable association with a protein to trigger an immune response and cause allergic sensitization (Kimber et al., 2011).

Chemical respiratory allergy resulting in rhinitis and asthma is an important occupational health issue. It is associated with high levels of morbidity and has significant financial costs. In contrast to skin sensitization and allergic contact dermatitis, where many hundreds of chemicals have been confirmed as causative agents, there are less than 100, and probably no more than 80, chemicals that have been implicated as having the potential to cause allergic sensitization of the respiratory tract. Among these are the diisocyanates, chlorplatinate salts, acid anhydrides, and some reactive dyes (Kimber et al., 2014b).

It has commonly been assumed that inhalation exposure is necessary for respiratory sensitization and elicitation. However, there is evidence from both experimental studies and clinical observations that effective sensitization of the respiratory tract can result from skin contact with chemical respiratory allergens. In susceptible individuals, dermal exposure to a chemical respiratory allergen can result in the class of immune response required for respiratory sensitization (Rattray et al., 1994; Arts et al., 1998; Kimber and Dearman, 2002; Kimber et al., 2014b). The relevance of skin exposure for respiratory sensitization to chemicals is an important consideration when interpreting data from sensitization studies in experimental animals.

A significant challenge to assess respiratory sensitization has been the absence of methods for the identification and characterisation of chemical respiratory allergens. Despite research for more than three decades, there are still no validated methods for the assessment of the respiratory sensitizing potential of LMW chemicals. Indeed, there are not even approaches that are widely recognised within the scientific community, or by regulatory agencies. Partly this is due to continuing uncertainty regarding the immunological mechanisms through which sensitization is acquired, and in particular about the role of IgE antibody (Kimber et al., 1998, 2014a; 2014b; Kimber and Dearman, 2002). However, there is a growing consensus about the cellular requirements: contact allergy is closely associated with the elicitation of selective T helper (Th)-1 type immune responses whereas chemical respiratory allergy is closely associated with T helper (Th)-2 type immune responses. This is based on experimental studies in which chemical respiratory allergens have been found to induce preferential Th2-type T cell responses (Dearman et al., 1995, 1996; 2005; Van Och et al., 2002; Kimber et al., 2011) and there are data indicating that a similar Th2 selectivity is found in humans also (Ouyang et al., 2013; Newell et al., 2013; Kimber et al., 2014c). See for more information on the mechanism of type 2 immunopathology e.g. the paper of Samuchiwal and Boyce (2018).

In the absence of (a) validated and universally recognised test(s) for the identification of chemical respiratory allergens, what can be done instead? The following main sources of information might inform decisions about the presence or absence of respiratory sensitizing potential: clinical observations and investigations in workers, and experimental (animal) studies including *in silico*, *in chemico* and *in vitro* testing. First the clinical observations and investigations in workers will be addressed.

3. Clinical observations and investigations

Information on LMW chemicals inducing respiratory allergy can be obtained from workplace studies in which exposed workers are investigated, from incidence data in national registers, and from case reports. Sensitization studies in volunteers are usually not performed (nor allowed in the EU) as these individuals may run the risk of becoming sensitized and develop respiratory allergy upon a subsequent exposure to that chemical.

The appearance of respiratory symptoms in workers during or after manufacturing may possibly point at occupational asthma but this alone is not sufficient for the diagnosis of allergic asthma. Occupational asthma can either be (a) sensitizer-induced asthma (allergic asthma), (b) irritant-induced asthma (non-allergic asthma, including Reactive Airways Dysfunction Syndrome (RADS)), or (c) work-exacerbated asthma (pre-existing asthma unrelated to work that is made worse by a workplace exposure; Arts and Kimber, 2017).

In order to confirm a diagnosis of allergic occupational asthma the following criteria must be met: (1) diagnosis of asthma by a physician, (2) a proven association between the symptoms of asthma and work, (3) workplace exposure to an agent or process previously associated with work-related asthma, and at least two of the following criteria: (4) significant work-related changes in spirometry, (5) significant work-related changes in non-specific airway hyperresponsiveness, and (6) a positive response to an inhalation provocation with the specific agent to which the individual is exposed at work (Klees et al., 1990).

In many worker studies associations between symptoms of asthma and work have been reported, but very often lung function tests and/or non-specific airway hyperresponsiveness and/or inhalation provocation tests with the suspected chemical (without impurities) have not been conducted. Additional immunological measurements are of considerable value in confirming a diagnosis of allergic asthma, as even with the six criteria mentioned it is difficult to discriminate between occupational asthma of types (a), (b) and (c). However, this is also not frequently done. In addition, information on atopy or predisposing factors is generally not or only partly available in case reports, and it is mostly absent in worker studies and for the cases included in the national registers. Also, bias may play a role: if one of the chemicals to which workers have been exposed has already been classified as a respiratory sensitizer, it will be difficult not to conclude it is that chemical that has caused the occupational asthma with as consequence that no further investigations will be done. And in how far can co-exposure to other substances be excluded during workplace exposure(s) (Arts and Kimber, 2017)?

Indeed, many work-related asthma cases may not be associated with exposure to allergens. Reinisch et al. (2001) reported that of the new asthma cases in California in 1993–1996, approximately one third (35%) of these new cases were identified as work-aggravated asthma. Individuals with predisposing risk factors for asthma, atopy and a family history of asthma are likely to first develop asthma due to non-work exposures, but then experience work-related exacerbation of pre-existing asthma. The other 65% consisted of new-onset work-related asthma cases. However, of these new-onset cases, only 13.4% was associated with exposure to a previously documented allergen, 77.2% was associated with exposure to agents that were unknown to be allergens, and 9.3% was based on an irritant mechanism. Also, for methyl methacrylate (MMA) cohort and cross-sectional worker studies reported irritation of eyes, nose, and upper respiratory tract associated with short-term peaks exposures, but little evidence for respiratory sensitization or asthma. Nineteen case reports described asthma, laryngitis, or hypersensitivity pneumonitis in MMA-exposed workers; however, exposures were either not well described or involved mixtures containing more reactive respiratory sensitizers and irritants. The authors indicated that the weight of evidence is not sufficient to conclude that MMA would be a respiratory sensitizer (Borak et al., 2011).

Thus, considering all available information presented in such

worker studies collectively, it is very difficult to conclude with certainty that a specific chemical has the potential to induce immunologically mediated respiratory allergy in humans.

4. Experimental studies

4.1. Animal testing

Several approaches have been taken to asses respiratory sensitization in test animals. Overviews have been presented by Arts and Kuper (2003, 2007), and more recently by Chary et al. (2018). In short, these approaches can be divided in sensitization tests such as the serum IgE test and cytokine fingerprinting, and sensitization plus elicitation tests using a single or repeated challenge exposures.

In analogy to the dermal Local Lymph Node Assay (LLNA), the respiratory LLNA was developed. Rather than applying the test material onto the skin of mice for three consecutive days, mice were exposed by inhalation head/nose-only to the test material during three consecutive days for 45, 90, 180 or 360 min/day. Ear application (skin LLNA) was used as a positive control. Negative controls were exposed to the vehicle. Three days after the last exposure, proliferation was determined in the draining mandibular lymph nodes, and the respiratory tract was examined microscopically. In the respiratory LLNA both typical contact and respiratory allergens tested positive (Arts et al., 2008) and both types of sensitizers could be identified by different cytokine profiles (De Jong et al., 2009). However, formaldehyde (Arts et al., 2008) and glutaraldehyde (Van Triel et al., 2011) tested negative as they did not induce a Stimulation Index (SI) $\geq$ 3 (a 3-fold or greater increase in proliferation (thymidine incorporation) compared with vehicle controls). It was first suggested that the tested formaldehyde (FA) concentration (3 ppm) may not have been sufficiently high per unit surface area, although slight irritation of the upper respiratory tract was seen. Glutaraldehyde (GA) was therefore tested at higher levels, viz. at 6 and 18 ppm, and both as vapour and aerosol, and distinct irritation was seen in the upper respiratory tract. It was hypothesized that the highly reactive and hydrophilic GA oligomerized in the lysine-rich protein mucous layer of the respiratory tract, thereby preventing GA from reaching the antigen presenting cells (APCs) in sufficient quantities to induce sensitization but still facilitating local irritation, and that the mucous lining of the airways may also protect against respiratory sensitization by FA as FA has solubility and reactivity characteristics similar to GA. The skin has no such aqueous mucous lining which may explain the positive results of FA and GA in the dermal LLNA (Van Triel et al., 2011).

The dermal LLNA has also been combined with gene expression analysis. Typical respiratory sensitizers such as trimellitic anhydride (TMA) and ortho-phthalaldehyde could be distinguished by differences in gene expression responses from the typical dermal sensitizers 2,4-dinitro-1-chlorobenzene (DNCB) and hexylcinnamic aldehyde (HCA), and from the non-sensitizing irritants methyl salicylate and nonanoic acid (Boverhof et al., 2009; Adenuga et al., 2012).

It was also noted that reactions upon dermal sensitization and inhalation challenge with TMA and DNCB differed strongly in high IgE responding BN rats versus low IgE responding Wistar rats (Arts et al., 1998). In addition, it turned out to be possible to discriminate between respiratory allergic and respiratory irritation reactions (Arts and Kuper, 2003) and finally, the dermal sensitization route was to be preferred over inhalation sensitization, because of stronger reactions and because respiratory irritation may confound the allergic airway reactions (functional breathing responses as well as histopathologically).

Investigations of dose-response relationships and/or threshold levels in respiratory allergy were reviewed by Arts et al. (2006) and later by Cochrane et al. (2015).

However, although progress has been made (Botham et al., 1988; Griffiths-Johnson and Karol, 1991; Sarlo and Clark, 1992; Satoh et al., 1995; Hilton et al., 1996; Dearman and Kimber, 2001; Arts et al., 2008; Pauluhn, 2008; Lalko et al., 2011), none of the respiratory sensitization tests has yet resulted in a standard approach that can be used with confidence for the identification of respiratory sensitizers and as such has been included in an official OECD guideline. Besides the earlier mentioned uncertainty regarding the immunological mechanism involved, other reasons may be lack of time, resources, the need to set up a validation round with other labs, and thus costs, and the need to operate an inhalation facility. Nevertheless, in view of the increasing reluctance to perform animal tests, the set up and validation of an OECD respiratory sensitization and challenge testing guideline in animals most probably is too late.

In the absence of validated methods for the identification of chemical respiratory allergens, one strategy has been to examine the behaviour of chemical respiratory allergens in test methods for the evaluation of skin sensitization potential such as the dermal LLNA in mice and rats (Kimber and Weisenberger, 1989; Kimber and Basketter, 1992; Kimber et al., 1994, 2002; Arts et al., 1996; Basketter et al., 2002), and tests in guinea pigs such as the GPMT (Magnusson and Kligman, 1969) and the occluded patch test (Buehler, 1965). According to Dearman et al. (2013), with only a single exception - piperazine - chemical respiratory allergens (such as acid anhydrides, amines, aldehydes, isocyanates, platinum salts, reactive dyes and uronium salts) also tested positive in these mouse and guinea pig test methods. However, piperazine showed signs of skin sensitization in 5 out of 20 animals (25%), just below the 30% limit used for classification (Leung and Auletta, 1997).

The LLNA is predicated on the fact that skin sensitizing chemicals will induce lymphocyte (primarily T cell) activation and proliferation in regional lymph nodes draining the site of skin exposure (Kimber et al., 2011). Chemicals that at one or more test concentrations elicit an SI $\geq$ 3 are classified as skin sensitizers (Kimber et al., 2002). Several hundreds of chemicals have been tested in the LLNA (Kern et al., 2010) and, in addition to skin sensitizers, all known chemical respiratory allergens that have been tested in the LLNA elicited positive responses (Dearman et al., 2013).

Although both contact allergens and respiratory allergens tested positive in the LLNA, it is known that they induce different qualities of T cell response. Chemical respiratory allergens elicit selective Th2-type responses, whereas contact allergens are associated with preferential Th1 responses (Dearman et al., 1995, 1996; 2005; Van Och et al., 2002; Kimber et al., 2011). However, such differential qualities of T cell response are not registered in the standard LLNA (OECD 429) which simply measures the proliferation of lymph node cells. Although the characteristics of immune responses elicited by contact allergens and respiratory allergens differ qualitatively, they are both associated with the activation and proliferation of T lymphocytes in lymph nodes draining the site of exposure. For this reason, chemical respiratory allergens and contact allergens elicit positive responses in the LLNA when administered topically. It is after that initial activation of responsive T lymphocytes that the immune responses induced by contact allergens and chemical respiratory allergens begin to diverge in a qualitative sense (Cochrane et al., 2015). And that's why typical contact and respiratory allergens also tested positive in the respiratory LLNA (Arts et al., 2008) as indicated above.

In addition, whereas the standard dermal LLNA is based on the evaluation of immune responses induced following skin contact, it is now established that the skin is a relevant route of exposure for sensitization of the respiratory tract to chemicals (summarized in Kimber and Dearman, 2002; Redlich and Herrick, 2008; Redlich, 2010; Kimber et al., 2014b). Thus, there is good reason for chemical respiratory allergens to induce T cell activation and proliferation in regional lymph nodes following skin exposure, and to elicit positive responses in the LLNA. Moreover, it is assumed that respiratory allergens also elicit positive responses in guinea pig assays for the same reason; that they provoke T cell responses that drive skin reactions following challenge of sensitized animals.

Based on this mechanistic activity of chemical respiratory allergens in the LLNA (in mice and rats), and in guinea pig assays, it has become apparent that this activity provides a useful tool for examining the respiratory sensitizing potential of chemicals: chemicals that fail to elicit positive responses in the LLNA can therefore be regarded as lacking not only skin sensitizing activity, but also the potential to induce sensitization of the respiratory tract. That is, chemicals that test negative in the LLNA (and/or guinea pig tests) can be eliminated with confidence from further consideration as possible chemical respiratory allergens (Dearman et al., 2013). Bloemen et al. (2009) listed several other LMW chemicals as respiratory sensitizers based on human case studies showing these chemicals had caused occupational asthma. Based on differences in the outcome of LLNA results it could lead to the assumption that the LLNA would be of only limited predictive value for the absence of respiratory sensitization. Of course, the LLNA is no golden standard but it should be noted that LMW chemicals currently classified (or listed) as respiratory sensitizers only based on human clinical observations may be asthmagens and no respiratory allergens. Most likely this is the reason that the LLNA would not be of predictive value in case of assessment of asthmagens. Nevertheless, Dik et al. (2014) included 168 chemicals that tested negative in the LLNA in their database as respiratory non-sensitizers.

Although the LLNA (OECD 429) is a sensitization test - so no induction of clinical symptoms in animals as no subsequent elicitation/provocation test is performed - it is still an animal test which, according to the latest EU REACH requirements, has resulted in the need to test for skin sensitization *in vitro* first. However, in case some of these *in vitro* tests are positive, or in case not all 3 *in vitro* tests can be carried out (e.g. due to solubility issues), a LLNA may still need to be performed.

4.2. In silico/in chemico/in vitro testing

4.2.1. In silico sensitization testing

In silico testing can consist of using (Q)SAR (Quantitative Structure-Activity Relationship) models (expert models, knowledge based using structural fragments) and/or e.g. OECD QSAR toolbox (which includes read across, trend analysis and QSAR models; Patlewicz et al., 2013). However, neither a full positive nor a full negative will be obtained, and as such *in silico* analysis on its own will not be sufficient for correct classification. In addition, a (Q)SAR analysis is as good as its database is. For skin sensitizers there is a large database because many chemicals have been tested in dermal sensitization tests and/or for which human data are available. However, even here there may be doubt as f.i. positive results in the LLNA cannot always be used, such as in the case of surface-active substances or irritants which have resulted in false-positive outcomes (Kreiling et al., 2008; Basketter et al., 2009; Garcia et al., 2010; Ball et al., 2011). Taking this into account for skin sensitizers, and in view of the absence of validated tests for respiratory sensitizers, how accurate would the current database for respiratory sensitizers be? Can we be confident that all respiratory sensitizers listed are indeed respiratory sensitizers/allergens and not asthmagens/irritants?

As an example: ADCA (CAS no. 123-77-3) has officially been classified as a respiratory sensitizer but is negative in the dermal LLNA, GPMT, and Buehler test, and does not show hypersensitivity reactions in guinea pigs in a 28-day inhalation study in which also a challenge exposure had been included. Although the latter study is not a standard test, other studies with known respiratory sensitizers were positive. Also, symptoms reported in workers are far from conclusive (summarized in Arts and Kimber, 2017). Profiling using the OECD QSAR Toolbox (version 4.3) learns that ADCA could interact with proteins (lysine or cysteine) via a Michael addition mechanism (Hill and Vederas, 1999); chemicals with such a structural alert would be assessed in the Toolbox as respiratory sensitizer category 1A. However, according to Kimber et al. (2018) there are two features that are commonly identified as being associated with chemical respiratory allergens: the first is 'hard' electrophilic activity and the second is an ability to cross-link proteins. Enoch et al. (2010) included ADCA in their SAR as it was listed as a respiratory sensitizer by the UK HSE and used as a rationale that ADCA is a very reactive Michael acceptor and therefore it would likely be sufficiently reactive towards lysine to cause respiratory sensitization. However, information on cross-linking potential is lacking. It was also noted in the Toolbox that the dataset from which the profiler was developed contained only a single chemical that featured this structural alert, viz ADCA itself, based on some observations in humans (Slovak, 1981; Normand et al., 1989; Kim et al., 2004).

Using Derek (version Derek Nexus 6.0.1; Nexus 2.2.1), which has rules for respiratory sensitization, ADCA pops up as an alert (alert 282) for plausible occupational asthma based on some same and other observations in humans (Ferris et al., 1977; Slovak, 1981; Malo et al., 1985; UK HSE, 1997), and most likely because of its official classification. Interestingly, however, ADCA has been included in Derek in the category 'occupational asthma'[1] and not in the category 'respiratory sensitization', but it is not clear whether this could be considered being equivalent to an 'asthmagen' versus 'respiratory allergen'. On the other hand, Derek predicts ADCA to be a non-sensitizer with regard to skin sensitization but remarks in addition that it contains 'unclassified features' (structural features that do not appear in the negative prediction dataset; or in other words, the prediction is negative as none of the features of the chemical structure appear in the positive prediction set).

Dik et al. (2014) evaluated existing *in silico* models for the identification of respiratory sensitization, viz. MultiCASE (Graham et al., 1997), cat-SAR (Cunningham et al., 2005) and a logistic regression model (Jarvis et al., 2005). In addition to these three SAR models, they added two sets of structural alerts: the combined respiratory sensitization and occupational asthma knowledge database of Derek and a set of alerts proposed by Enoch et al. (2012). The predictive performance of the SARs calculated from the training sets was compared to their performance on a dataset of newly identified respiratory sensitizers and non-sensitizers derived from literature. In total, a dataset of 138 respiratory sensitizers and 521 non-sensitizers was compiled. Remarkably, although the authors emphasized that not all asthmagens induce immunological mediated hypersensitivity, they assumed for the purpose of their investigation that all occupational asthmagens are also respiratory allergens.

As none of the single SAR models was considered sufficiently reliable a tiered approach was suggested by Dik et al. (2014) combining the two SARs with the highest positive and negative predictivity taking into account model specific chemical applicability domain issues. For only 26 of the 138 respiratory sensitizers (19%) all models gave a positive prediction, and for 76 of the 521 respiratory non-sensitizers (15%) all models agreed on a negative prediction. The tiered approach by Dik et al. (2014) provided reliable predictions for 35% of the respiratory sensitizers and non-sensitizers compiled (with a positive and negative prediction value of 96% and 89%, respectively) but it was not able to predict the other 65% of the chemicals.

With regard to ADCA, the example mentioned above, this chemical was included in all five datasets as a structural alert and as such came out positive in the tiered approach by Dik et al. (2014). However, because a reason why chemicals have been included consists of 'a case of occupational asthma was reported in a peer-reviewed report', it can be questioned whether it was wise to consider all occupational asthmagens as respiratory allergens. Also, according to Kimber et al. (2014b) there are probably no more than 80 chemicals that have been implicated as having the potential to cause allergic sensitization of the respiratory tract. This number is substantially lower than the 138 used by Dik et al. (2014) who finally concluded that based on the 65% that could not be predicted there is an urgent need for other test methods (*in chemico/in*

[1] Note in Derek: Examples of active compounds which fire the alert: azodicarbonamide – no other examples are known.

vitro) to reach a reliable conclusion.

According to Arts and Kimber (2017), ADCA does not have the ability, under physiological conditions, to form sufficiently stable associations with proteins and is not a true respiratory allergen. There will certainly be more examples in view of the discussion above and in order not to pollute any QSAR database, for each LMW chemical currently classified as a respiratory sensitizer, a careful (re-)examination should be made on its potential respiratory allergenicity before inclusion in a database for true respiratory sensitizers.

4.2.2. *In chemico/in vitro sensitization testing*

Because respiratory sensitizers tested in assays for skin sensitization have tested positive in such tests it may be worth to first regard the key biological events underlying skin sensitization which have been summarized in the form of an Adverse Outcome Pathway (AOP), starting with the molecular initiating event through intermediate events to the adverse effect, namely allergic contact dermatitis:

The molecular initiating event (i.e. the first key event) is the covalent binding of electrophilic substances to nucleophilic centres in skin proteins. This can be measured by the Direct Peptide Reactivity Assay (DPRA) or the Amino acid Derivative Reactivity Assay (ADRA) which are included in OECD testing guideline 422C. The second key event takes place in the keratinocytes and includes inflammatory responses as well as changes in gene expression associated with specific cell signalling pathways such as the antioxidant/electrophile response element (ARE)-dependent pathways. There are two keratinocyte activation assays viz. the KeratinoSens and the LuSens method (OECD 442D). The third key event is the activation of dendritic cells (DC), typically assessed by expression of specific cell surface markers, chemokines and cytokines. One of the test methods is the Human Cell Line Activation Test (h-CLAT) included in OECD testing guideline 442E. The h-CLAT method quantifies changes in the expression of cell surface markers (CD86 and CD54) associated with the process of activation of monocytes and dendritic cells (DC) in the human monocytic leukaemia cell line THP-1 following exposure to sensitizers. The measured expression levels of CD86 and CD54 are then used for supporting the discrimination between skin sensitizers and non-sensitizers. The fourth key event is T-cell activation and proliferation, which is indirectly assessed in the murine Local Lymph Node Assay (LLNA; see above).

An AOP has also been described for respiratory sensitization (Sullivan et al., 2017) consisting of: (1) covalent binding of chemicals to proteins, (2) activation of cellular danger signals (inflammatory cytokines and chemokines and cytoprotective gene pathways), (3) dendritic cell activation and migration, (4) activation, proliferation, and polarization of T cells, and (5) sensitization of the respiratory tract. These events mirror the skin sensitization AOP but with specific differences: respiratory sensitizers bind preferentially to lysine moieties, whereas skin sensitizers bind to both cysteine and lysine, and exposure to respiratory sensitizers seems to result in skewing toward cytokine secretions predominantly associated with T helper 2 (Th2) response; see for extended reviews Sullivan et al. (2017) and Samuchiwal and Boyce (2018). Except for the first step (protein binding) for which the DPRA can be used and step 4 for which the LLNA could be used, no specific OECD TGs are as yet available for step 2 and 3.

In the DPRA, chemical respiratory sensitizers – being hard electrophiles - have shown a preferential reactivity with lysine peptides which may result in different protein conjugates and thus influence the immune response (Lalko et al., 2012). However, the word 'preferential' indicates it is not conclusive. Dik et al. (2016) modified the DPRA protocol to identify respiratory sensitization potential by including two peptide depletion measurement time points and added high-performance liquid chromatography mass spectrometry analysis of reaction products, which improved predictive capacity for sensitization; however, this adapted assay could not be used to distinguish respiratory from skin sensitizers. In the review by Chary et al. (2018) it was indicated that typical respiratory allergens such as maleic anhydride (MA), phthalic anhydride (PA), trimellitic anhydride (TMA), and toluene diisocyanate (TDI) all tested positive in the dermal LLNA. However, TMA was negative in the DPRA, PA was negative in the h-CLAT assay (using both CD86 and CD54 cell surface markers), whereas MA was negative in the h-CLAT assay using CD86 surface markers. TDI was positive in the DPRA but not tested in the h-CLAT assay, so there is currently too little information to also consider the existing OECD 442CDE tests, including the DPRA and h-CLAT assays, as a method to identify respiratory sensitizers.

During the last years, a few *in vitro* methods have been developed to study respiratory sensitization of LMW chemicals, generally focusing on one of the key events in the process of respiratory sensitization (Huang et al., 2013; Dik et al., 2015; Hermanns et al., 2015; Mizoguchi et al., 2017). An overview has been prepared by North et al. (2016); in the next section, a few *in vitro* methods are described in more detail:

Advances have been made *in vitro* to provide a realistic way for exposure of epithelial cells at the air-liquid interface (ALI). Because the respiratory tract is a complex organ with many cell types, it should be questioned which cells to include to study respiratory sensitization *in vitro*. Cells from the alveolar region could of course be used as they may be exposed (Roggen et al., 2006); however, in case of aerosol exposure (dust and droplets) the area of deposition may be in the upper respiratory tract only, depending on the particle size distribution. In the respiratory LLNA (Arts et al., 2008) using various skin and respiratory sensitizers, histopathological changes were only observed in the nasal area and larynx following a 3-day inhalation exposure regimen to vapours and respirable aerosols but not in the lungs which suggests the alveolar region had not been reached, or at least not by sufficient amounts.

A co-culture system for the detection of respiratory sensitizers has been developed by Chary et al. (2018, 2019) consisting of different cell types, viz. alveolar type II cells secreting lung surfactant, endothelial cells, dendritic cells and macrophages. The respiratory sensitizers TMA and PA were tested and the respiratory irritants acrolein and methyl salicylate served as negative control. They were tested at maximum 25% of cytotoxicity. Exposure to TMA and PA resulted in dendritic cell activation (TSLPr and CD54 cell surface markers expression) and a specific cytokine release pattern (upregulation of IL-10, GM-CSF and CCL20) whereas the irritants did not. The authors claimed that with this procedure – measuring cytokines at the alveolar barrier and activation of dendritic cells – 2 out of the 4 key events of the AOP for respiratory sensitization can be investigated (Chary et al., 2019). So far skin sensitizers have not been tested in this set up, and only a very limited number of respiratory sensitizers belonging to the acid anhydrides chemical group only. As such it is not known whether this system would be suitable to distinguish respiratory sensitizers from skin sensitizers.

The SENS-IS assay, currently under ECVAM validation and included in the OECD guideline preparation schedule, is an *in vitro* assay that aims to predict skin sensitization potency of chemical ingredients with a similar accuracy as the LLNA (although the LLNA cannot be considered a golden standard either as indicated previously). The assay uses human 3D epidermis as test system; the read-out uses the genomic signature measured by multi-target quantitative RT-PCR. According to the developers, the assay would have an accuracy of more than 90% compared to the LLNA and human data, not only for hazard assessment but also for classification of potency, viz. Cat 1A or Cat 1B. Because it uses human 3D epidermis, it would be possible with this assay to evaluate the hazard of a wide variety of chemicals regardless their solubility (Cottrez et al., 2016; Petry et al., 2018). However, so far it is not known whether in this assay typical respiratory sensitizers could be differentiated from typical dermal sensitizers.

In the *in vitro* Genomic Allergen Rapid Detection (GARDskin) test, instead of looking at common events such as total T-cell proliferation or upregulation of CD86 cell surface markers, this test aims to look directly at the mechanistic differences induced by skin-sensitizers and non-sensitizers on the transcriptomic level, using 200 genes. The test

was set up using a comparison between skin-sensitizers and non-sensitizers (Johansson et al., 2011, 2013), and also potency assessment has been claimed (Zeller et al., 2017). However, as indicated before, it is important to know whether the response from known respiratory allergens would be different from that of known dermal allergens in this assay. The developers of this test are currently collecting evidence that this assay does not falsely classify 'pure' respiratory sensitizers as skin sensitizers. F.i. phthalic anhydride was correctly classified as a non-sensitizer in GARDskin as well as a respiratory sensitizer in GARDair (The personal communication was with J. Schmidt, working at SenzaGen and took place in 2019). In the GARDair assay, the 28 genes selected are associated with the regulation of the TSLP pathway which is known to participate in T-cell polarization towards Th2, representing one of the key mechanistic events of allergic asthma. The test was set up with 10 known respiratory allergens and 20 non-respiratory sensitizers, the latter group including both irritants as well as skin sensitizers (Forreryd et al., 2015). Out of the 10 respiratory sensitizers 9 were correctly classified as such (except for TDI), whereas 7 out of 8 skin sensitizers (except for glyoxal) were correctly classified as non-respiratory sensitizers (The personal communication was with J. Schmidt, working at SenzaGen and took place in 2019). Although there seems to be high sensitivity, it is quite worrying that a potent respiratory sensitizer as TDI came out negative. Based on expected differences in cellular responses between the 3 groups (skin sensitizers, respiratory sensitizers, and irritants), it is very important to know whether these 3 groups can indeed be discriminated in both assays. So, when using the assays in combination, there are indications that the different responses can be distinguished but the two assays clearly need further investigations also in view of chemicals that are not so potent or poorly soluble (but it is fair to say this also applies to the *in vivo* tests).

5. Discussion and conclusion

The appearance of respiratory symptoms in workers in manufacturing or processing factories may possibly point at occupational asthma. However, this alone is not sufficient for the diagnosis of allergic asthma. As stated previously, the term occupational asthma embraces sensitizer-induced asthma (allergic asthma), irritant-induced asthma (non-allergic, including Reactive Airways Dysfunction Syndrome (RADS)), or work-exacerbated asthma (pre-existing asthma unrelated to work that is made worse by a workplace exposure). In order to confirm a diagnosis of allergic occupational asthma several criteria must be met (Klees et al., 1990).

In contrast to classification of skin sensitizers (H317; may cause an allergic skin reaction), classification of respiratory sensitizers (H334) according to GHS/CLP is not clear-cut. Any chemical that induces a specific respiratory hypersensitivity reaction at a low to moderate (cat. 1B) or high (cat. 1A) frequency in humans should, according to the criteria, be considered a respiratory sensitizer. However, low to moderate and high frequencies are not defined. Also, according to the criteria it is necessary to take into account the size of the exposed population and the extent and conditions of exposure, but this is not further clarified or explained. And finally, the word 'specific' is not clear as it can denote a response to a particular (specific) test substance or a specific immunological reaction (an immunological reaction specific for the substance); however, immunological mechanisms do not have to be demonstrated.

The guidance further indicates that appropriate lung function tests or bronchial challenge tests should be conducted according to accepted guidelines in order to provide evidence for respiratory sensitization. It is relevant to consider, therefore, whether any lung function tests conducted are appropriate and performed according to accepted guidelines using a pure form of the chemical under investigation. In addition, occupational, medical and smoking histories should be known. Also, according to the guidance, symptoms of asthma by irritation do not apply for classification in case of existing bronchial hyperreactivity. If it can be demonstrated that these substances induce symptoms of asthma by irritation only in people with bronchial hyperreactivity, they should not be considered respiratory sensitizers. However, if only non-specific bronchial hyperreactivity tests will be carried out, it cannot be concluded nor excluded whether a specific chemical induced symptoms of asthma by irritation (Arts and Kimber, 2017).

In addition, in order not to pollute any QSAR database assessing respiratory sensitization, for each LMW chemical currently classified or listed as a respiratory sensitizer, a careful (re-)examination of its potential respiratory allergenicity should be done before inclusion in that database.

There are no validated *in vivo* animal models to assess respiratory sensitization of LMW chemicals, and most probably no effort will be put in validating any of the existing approaches in view of 'animal testing being the last resort'. Nevertheless, LMW chemicals that have tested negative in *in vivo* assays for skin sensitization should be considered to also lack the potential to induce respiratory sensitization (Arts and Kimber, 2017). Chemicals that have tested positive in such assays could either be a skin sensitizer or a respiratory sensitizer as these assays do not really allow to discriminate skin from respiratory sensitizers (Chary et al., 2018), which in case of a positive test also leads to the question whether classification as skin sensitizer would always be correct.

So for any new LMW chemical to be tested the hope is to have at least one appropriate, predictive and cost-effective *in vitro* assay (or a combination thereof) to assess respiratory sensitization. Efforts have been made using potentially relevant cell types and exposure conditions such as ALI, or to investigate differences in cell surface marker expression and cytokine production. However, it could be questioned whether lung tissue, lung epithelia/endothelia and ALI exposure are the most appropriate to investigate an allergic disease characterized by rhinitis and bronchoconstriction. And can elicitation be captured *in vitro*? Perhaps gene profiling may offer a solution, but further effort is needed to identify respiratory sensitizers and to distinguish these from skin sensitizers and irritants, not at least because of the far-reaching consequences once classified as respiratory sensitizer.

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Indoor air chemistry: Terpene reaction products and airway effects

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ABSTRACT

Reactive chemistry is ubiquitous indoors with a wealth of complex oxidation reactions; some of these are initiated by both homogeneous and heterogeneous reaction of ozone with unsaturated organic compounds and subsequent the hydroxyl radical, either in the gas-phase or on reactive surfaces. One major focus has been the reaction of common and abundant terpene-based fragrances in indoor air emitted from many wood-based materials, a variety of consumer products, and citrus fruits and flowers. Inhalation of the terpenes themselves are generally not considered a health concern (both acute and long-term) due to their low indoor air concentrations; however, their gas- and surface reactions with ozone and the hydroxyl radical produce a host of products, both gaseous, *i. a.* formaldehyde, and ultrafine particles formed by condensation/nucleation processes. These reaction products may be of health concern. Human cell bioassays with key reaction products from ozone-initiated terpene reactions have shown some inflammatory reactions, but results are difficult to interpret for human exposure and risk assessment. Acute effects like sensory irritation in eyes and airways are unlikely or present at very low intensity in real life conditions based on rodent and human exposure studies and known thresholds for sensory irritation in eyes and airways and derived human reference values for airflow limitation and pulmonary irritation. Some fragrances and their ozone-initiated reaction products may possess anti-inflammatory properties. However, long-term effects of the reaction products as ultrafine particles are poorly explored. Material and product surfaces with high ozone deposition velocities may significantly impact the perceived air quality by altered emissions from both homogeneous and heterogeneous surface reactions.

1. Introduction

In the search for eye and airway irritants (mucous membrane irritation) in office-like environments it was realized that concentration levels of typical volatile organic compounds (VOCs) and formaldehyde were far below thresholds for sensory irritation to account for reported eye and airway symptoms (Wolkoff et al., 1997). Thus, the "reactive chemistry" hypothesis was created by the fact that common and abundant indoor fragrances, applied in numerous cleaning and consumer products, like mono-terpenes, undergo ozone-initiated reactions fast enough indoors to compete with the air exchange rate (AER) (Weschler and Shields, 1997, 2000). Initially, the hypothesis was suggested by Stirling and Stirling (1983), but it was later prompted by the observation that loss in total VOC raised the level of formaldehyde simultaneously with an increase in reported symptoms in offices (Sundell et al., 1993); Wolkoff (1995) further discussed these findings. The focus on these ozone-initiated reactions is further encouraged by the ground-level increase of ozone by the global climate change (Vingarzan, 2004; Fann et al., 2015).

The ozone-initiated reaction of terpenes produces a host of new oxygenated volatiles, e.g. formaldehyde (Calogirou et al., 1999; Atkinson and Arey, 2003), and of which some multi-oxygenated compounds (e.g. dicarbonyls, peroxides) condense forming ultrafine secondary organic aerosols (SOA), e.g. (Glasius et al., 2000; Walser et al., 2008). Some of the SOA may also adsorb/condense onto existing particles or nucleate. Ozone-initiated terpene reactions also produce the hydroxyl radical, which rapidly reacts further with VOCs; in general, to form a complex mixture of new oxygenated VOCs, see Fig. 1. Furthermore, hydrogen peroxide is formed in small amounts (Atkinson and Arey, 2003; Weinman et al., 2000) and secondary ozonides, e.g. Nørgaard et al. (2007). In view of this new hypothesis, considerable effort has been carried out to explore the airway toxicology of ozone-initiated terpene reactions by animal biossays, human lung cell studies, and human exposure studies.

Emission studies have also reported how exposure of building materials and treated surfaces to ozone alters their emission profile and may influence the perceived air quality (PAQ). Furthermore, skin-oils and other human debris may likewise react with ozone and produce new oxygenated compounds (Weschler, 2015).

The purpose of this overview is to try to discuss whether gas-phase ozone-initiated mono- and sesqui-terpene chemistry can cause acute eye and upper and lower airway (pulmonary) effects in office-like

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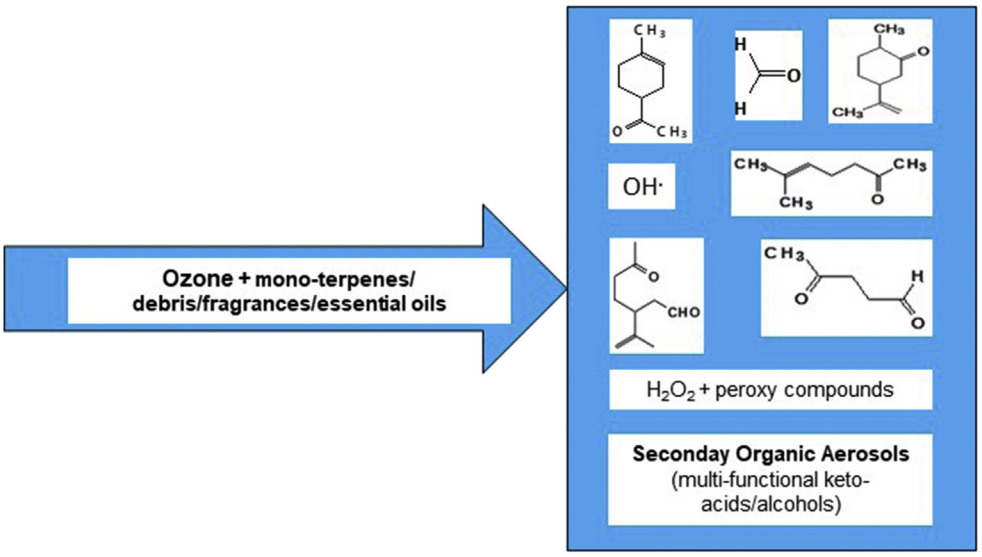

Fig. 1. Major oxidation products from ozone-initiated reactions with common mono-terpenes in fragrances, essential oils, and with human debris; for reaction with limonene see detailsin Figs. 1 and 2 in Carslaw (2013).

environments. Furthermore, briefly to review how terpene-related surface reactions may alter the PAQ. Thus, indoor chemistry in a broader perspective, as recently reviewed by Weschler and Carslaw (2018), is excluded from this overview. Thus, this overview is an extension and update of previous findings reported by Wells et al. (2017), Weschler (2015), Wolkoff (2013), and Wolkoff and Nielsen (2017).

2. Experimental studies

2.1. Modeling studies

Detailed mechanistic gas-phase model studies have been carried out. The studies apply real species and involving oxidation reactions of major indoor VOCs like limonene from use of a cleaning product in a residence with defined AER and work plan; furthermore, indoor lighting conditions are involved in the model. In the first study that involved 46 indoor VOCs estimated peroxy acetyl nitrate to be in the order of a few ppb at an AER of 2 h^{-1} (Carslaw, 2007). In a second study that mimicked a high ozone (50 ppb; 100 μg/m^3) cleaning simulation for 30 min with a maximum limonene concentration of 160 ppb (891 μg/m^3) resulted in total average concentrations of formaldehyde, 4-acetyl-1-methylcyclohexene (4-AMCH) and 3-isopropenyl-6-oxo-heptanal (IPOH) of 41, 23 and 13 μg/m^3, respectively, including about 3 μg/m^3 hydrogen peroxide (Carslaw, 2013). This study also predicts that SOA are dominated by peroxide and nitrated species, likely to condense onto existing particles.

During extreme climate conditions in European offices formaldehyde, 4-AMCH, IPOH, and 4-oxo-pentanal (4-OPA) were modeled at low/high AER and cleaning versus no cleaning in the morning or late afternoon by use of the detailed indoor air chemistry model using limonene as reactive model VOC. Average concentrations of formaldehyde, 4-AMCH and IPOH were found slightly higher by late afternoon cleaning (relative to morning cleaning) at 20, 17, and 19 μg/m^3, respectively, and 27 ppb indoor ozone (54 μg/m^3) at 1.5 AER (Terry et al., 2014). The predicted levels are above those measured in other European offices by Nørgaard et al. (2014a), see Table 1. Levels, that are more in line with the measured concentrations, were predicted from cleaning in the morning at 4.7 ppb (9 μg/m^3) ozone; this resulted in 8, 2.5, and 8 μg/m^3 of formaldehyde, 4-AMCH, and IPOH, respectively. 4-OPA was insignificant in both model studies, thus indicating other sources, which include surface reactions, cf. Nørgaard et al. (2014a) and Xiong et al. (2019).

Surface reactions on skin and breath in context of school classrooms have been modeled. About 20 ppb ozone decreases to 14 ppb, which estimated formic and acetic acid, and 4-OPA at levels of 0.8, 0.5, and 0.1 ppb, in the presence of pupils (Kruza and Carslaw et al., 2017). In a follow-up study, nonanal (5–7 ppb) was predicted to be the most important aldehyde derived from ozone-initiated surface reactions with painted walls in an apartment, and with predicted levels of nonanal, decanal and 4-OPA at 0.5, 0.7, and 0.7 ppb, respectively, from human skin reactions (Kruza et al., 2017); similar levels were found by Xiong et al. (2019). The concentration of the aldehydes, acids, and 4-OPA are far below any concern of acute eye and upper airway effects (Wolkoff et al., 2013, 2014; Nielsen, 2018).

2.2. Emission studies

Many studies have shown how exposure of building materials and consumer products to ozone produces a host of new oxygenated species, small acids, formaldehyde and longer-chain aldehydes, and ultrafine particles, see Salthammer and Bahadir (2009). The reactions are either homogeneous in the gas-phase or heterogeneous on surfaces. The classic study is the exposure of a carpet to ozone, which reacts with residual unreacted compounds in the latex backing (Weschler et al., 1992). Both formic and acetic acids emit from exposed latex paints (Reiss et al., 1995). The exposure of ozone to various building materials, insulation materials, and floor dust generally enhance the emission of longer-chain saturated aliphatic aldehydes (e.g., Nicolas et al. (2007); Poppendieck et al. (2007); Gall et al. (2013); Vibenholt et al. (2014); Chin et al. (2019)), including unsaturated aldehydes (e.g., Morrison and Nazaroff, 2002), keto-aldehydes from cleaning products (e.g., Singer et al., 2006), formaldehyde from essential oils (e.g., Huang et al., 2012), and ultrafine particles, e.g. Coleman et al. (2008b), Lamorena et al. (2006, 2008), Sarwar and Corsi (2007), Toftum et al. (2008), and Schripp et al. (2012). Heterogeneous surface reactions also add to the production of both new gas phase and SOA. For instance, reactions initiated on a cleaned PVC floor (Ham and Wells, 2011), materials and clothing, e.g. Coleman et al. (2008a), Palmisani et al. (2020), and Xiong et al. (2019), and skin-oils (Weschler, 2015).

Overall, ozone exposure will alter the composition of VOCs, either emitted from surface, present on the surface or coated on surfaces, thus, altering the PAQ, see below. Generally, the elevated concentration of produced VOCs, e.g. nonanal, will be one to three orders of magnitude below their thresholds for sensory irritation in eyes and airways

Table 1
Measured and modeled maximum concentrations (μg/m^3) in aircraft cabins, classrooms, modeling studies, and offices of limonene/terpene ozone-initiated reaction products, and guideline and threshold values for effects in eyes and airways.

Study	Limo nene	Formic acid	Acetic acid	Formalde-hyde	Acro lein	4-AMCH	IPOH	6-MHO	4-OPA	Ozone
Aircraft cabins										
Dechow et al. (1997)			30	35						
Pierce et al. (1999)				< 5	< 1.5					
Rosenberger et al. (2015)[a]				44	6					300
Wang et al. (2014b)[b]	660		23					21		
Weisel et al. (2013)								73		~160
Weschler et al. (2007)								34	39	~130
Classrooms										
Fischer et al. (2013)								1	3	75
Xiong et al. (2019)								4	2	64
Modeling										
Carslaw (2013)[c] (chemical box)	978			51		28	65			100
Carslaw et al. (2017) (classroom)				3		< 1	< 1			
Kruza and Carslaw (2017) (classroom)		1	1						4–6	
Kruza et al. (2017) (bedroom at night)									3	
[d]Terry et al. (2014) (offices)	1210			26		55	24			58
Offices										
Nørgaard et al. (2014a)	52			24		1	14	8	21	37
Salonen et al. (2009)	240		610[e]	44				4		
Wisthaler and Weschler (2010)[f]				9				12	8	66
Ventilation filters										
Destaillats et al. (2011)									47	
Guideline and threshold values	90,000[g]		5000[g]	100[g]	21[g]	1130[h]	1100[g]	1550[g]	123[h]	100[i]

[a] From 44 measurements in Airbus A321.
[b] Fourth quartiles concentration in 14 flights.
[c] Gas-phase modeled maximum concentrations after cleaning event, AER = 0.5 h^{-1} (Table 4).
[d] Peak concentrations after a late afternoon cleaning event in offices (Table 5; AER = 1.5 h^{-1}).
[e] Possibly, in part an analytical artifact.
[f] AER = 1 h^{-1}.
[g] Value for sensory irritation (Nielsen, 2018; Trantallidi et al., 2015; Wolkoff, 2013; Wolkoff and Nielsen, 2010; Wolkoff et al., 2013, 2014).
[h] Reference value for airflow limitation (Wolkoff et al., 2013, 2014, 2016).
[i] Pulmonary irritant (World Health Organization, 2006).

(Wolkoff, 2013). Thus, the AQ perception will be altered, but the change is unlikely to cause mucous sensory irritation symptoms in the eyes and upper airways.

2.3. Human lung cell effect in-vitro studies

The exposure of human bronchial epithelial cells (BEAS-2b) to magnetic nanoparticles coated with SOA generated from α-pinene or terpinolene showed minor elevation of IL-8, among many inflammatory markers; effects were absent for the SOA or the nanoparticles alone or clean air (Jang et al., 2006). Exposure of human lung epithelial cells (A549) to ozone-initiated non-denuded reaction mixtures with α-terpineol or limonene showed no biological effects at levels that mimic indoor air (Anderson et al., 2010, 2013).

In summary, the overall effect(s) of ozone-initiated terpene reaction mixtures, which included release of inflammatory markers, appear to be of minor importance for those investigated. Recently, specific ozone-limonene initiated reaction products have been tested for inflammatory effects and oxidative stress in human bronchial and alveolar epithelial cells (Lipsa et al., 2016, 2018). Unfortunately, opposite effects are observed between cell lines, and thus it is difficult to interpret and generalize; however, 4-OPA appears the strongest destructor of cell viability, while IPOH showed strongest potency for induced inflammation. It is, however, not possible to extrapolate the above results to real life conditions. 4-OPA as a strong destructor of cell viability may somehow be compatible with bronchoconstriction (airflow limitation) observed in exposed mice, see 2.4.

2.4. Animal exposure effect studies for health assessment

A number of acute airway effect studies of ozone-terpene reaction mixtures in rodents and humans have been reviewed (Rohr, 2013; Wolkoff and Nielsen, 2017). The major effects were sensory irritation in the upper airways with some minor effect observed in the conducting airways, while inflammation was not observed. For instance, bronchoalveolar lavage (BAL) in mice exposed repeatedly to ozone-initiated limonene oxidation products for 10 days showed no signs of inflammation and did not cause elevated development of airflow limitation or inflammation in the airways; sensory irritation was the major effect observed (Wolkoff et al., 2012). Based on the study, it was concluded that ozone < 200 μg/m^3 (0.1 ppm) would be safe for sensory irritation, even at high levels of limonene. About 75% of the sensory response could be assigned to formaldehyde and residual limonene (Wolkoff et al., 2008); however, moderate airflow limitation (bronchoconstriction) was also observed (Rohr et al., 2002; Wolkoff et al., 2008). The ozone-initiated limonene products in a reaction mixture showed no biological response from denuded SOA regarding sensory effects or airflow limitation (Wolkoff et al., 2008). In contrast to ozone alone, the ozone-limonene reaction mixture did not induce neither inflammatory nor genotoxic effects (Bornholdt et al., 2002).

In another study, F344 rats and ApoE−/− mice were exposed for seven days to denuded α-pinene-SOA derived from UV radiation of a mixture of nitrogen dioxide (+/− sulfur dioxide) and α-pinene (McDonald et al., 2010). Pulmonary inflammation was not observed in either mice or rats; the authors suggested the gaseous products to be of concern rather than the SOA. Furthermore, the biological response was

mild, including cardiovascular effects. In general, denuded SOA generated from 1.7 mg/m^3 (306 ppb) α-pinene and 1 mg/m^3 (510 ppb) ozone did not show clear pulmonary or systemic responses in rats according to Godelski et al. (2011) or *in vivo* oxidative stress (Lemos et al., 2011; Rohr and McDonald, 2016). The only significant finding was a minor increase of the breathing rate (Diaz et al., 2011); however, difficult to interpret.

Limonene may act as a scavenger for ozone and ROS (inflammatory mediators); for instance, as a local scavenger in the airways. Thus, an anti-inflammatory prophylactic effect of limonene alone has been shown in rodent inhalation models of allergic inflammation (Keinan et al., 2005; Hirota et al., 2012; Bibi et al., 2015) and also in a mice inhalation model for the ozone/limonene system (Hansen et al., 2013, 2016). Anti-inflammatory effects in lungs have also been suggested for the fragrance linalool (Huo et al., 2013) and other terpenes (Cho et al., 2017).

2.5. Human exposure studies for perceived air quality assessment

Although PAQ strictly is not directly associated with indoor air health, it is indirectly and may strongly affect not only well-being, but may also influence the perception of sensory irritation in eyes and upper airways; furthermore, PAQ may influence work performance, cf. Wolkoff (2013). Thus, PAQ is an essential element of the integrated assessment of indoor air health.

The ozone exposure of materials and products not only consume reactive VOCs but produce many new secondary VOCs and ultrafine products of which some may be of health concern, e.g. formaldehyde, and some may alter the PAQ, either by their different odor hedonics or low odor thresholds, e.g. unsaturated aliphatic aldehydes, cf. Wolkoff et al. (2006). In one study, a naïve sensory panel (n = 44) assessed in random order the emission of two specimen of the same preconditioned material of which one was exposed to a realistic ozone concentration (residual = 10 ppb; below odor threshold) and the other clean air (Knudsen et al., 2003). Out of eight typical indoor materials, the nylon carpet with latex backing showed a substantial difference in odor intensity, highest for the ozone exposed. Further, the sensory evaluation of the ozone-exposed carpet was strongly negative as reflected by a clear dislike. A similar observation was seen among female panel members, when assessing a carpet exposed to ozone in a climate chamber (Darling et al., 2012).

2.6. Human exposure studies for health assessment

Six human exposure studies have been carried out under controlled conditions in climate chambers. The studies aimed to explore both acute symptoms (sensory reactions) and inflammatory reactions in the airways. In the first one, young women (n = 130) were exposed to a typical indoor mixture with 23 VOCs (TVOC = 26 mg/m^3), including α-pinene (162 ppb, 0.9 mg/m^3) and limonene (126 ppb, 0.7 mg/m^3), for 140 min in a controlled climate chamber (25 m^3, 1.8 h^{-1}). The subjects' perception was masked by butyl acetate prior to the exposure. The mixture was used as such or mixed with ozone resulting in a residual concentration of 0.08 mg/m^3. No sign of inflammatory effects in nasal lavage was seen (Laumbach et al., 2005). The symptom rating was marginal and not statistically significant with or without ozone (Fiedler et al., 2005). The excess of VOCs may have scavenged the effects of the reaction mixture. Furthermore, the concentration of formaldehyde (40 μg/m^3) was too low to cause in sensory irritation.

In two studies, male subjects (n = 8) were exposed blindly in one eye for 20 min to a 10 min old ozone-limonene reaction mixture. The eye blink frequency of the subjects was video-recorded as a physiological measure of trigeminal stimulation of the eye (Klenø and Wolkoff, 2004, 2005). Mean blink frequency (mean of complete blinks for 4 min) increased significantly only during exposure to the reaction mixture compared with that of limonene or ozone alone, or sham air; the increase of the eye blink frequency coincided with the qualitative report of weak eye irritation symptoms. The initial concentrations of ozone and limonene were in the high-end, 130 and 220 ppb, respectively, and 20% relative humidity. The eye exposure concentrations were 40 ppb (80 μg/m^3) ozone and 75 (455 μg/m^3), respectively.

In the fourth study, young non-asthmatic subjects (n = 33) and mild asthmatics (n = 38) were blindly exposed for 3 h to a steady-state reaction mixture of maximum 74 μg/m^3 (36 ppb) ozone and 200 μg/m^3 (37 ppb) limonene in a climate chamber (240 m^3; 1 h^{-1}, recirculation 7 h^{-1}) (Fadeyi et al., 2015). The asthmatic subjects perceived significantly less nose and throat sensory irritation than the non-asthmatic subjects did. The rating was less than 15 on a continuous intensity scale from 0 to 100 with reported 20 = slight irritation. The difference between the non-asthmatic and asthmatic subjects is compatible with recent studies with naïve and sensitized mice exposed to formaldehyde or a reaction mixture of ozone and limonene indicating that "asthmatics" are less sensitive regarding sensory irritation in the airways (Larsen et al., 2013; Hansen et al., 2016). The differences in sensory eye irritation were insignificant; the difference was less than 13 on the intensity scale which is compatible with an expected formaldehyde concentration less than 50 μg/m^3 (40 ppb) (anticipated 20% reaction (Atkinson and Arey, 2003)), significantly lower than the threshold for sensory irritation in the eyes (Wolkoff and Nielsen, 2010). Furthermore, a stress marker (α-amylase) in saliva increased significantly in both the normal and asthmatic subjects after the exposure, but significantly more among the asthmatics. However, it is not clear whether the increase was caused by concern of the PAQ, the experimental set-up (asthmatics may have elevated stress level by the intense odor), or the reaction products. The odor of limonene must have been substantial in view of its odor threshold (Cain et al., 2007). The reported symptoms are in accordance with Wolkoff and Nielsen (2017).

In the fifth study, high frequency heart-rate variability (index of parasympathetic activity) was decreased with about 4% in healthy women (n = 22) exposed (double-blind) to a reaction mixture of limonene and ozone for 3 h in a controlled climate chamber (22 m^3). The initial/residual mean concentrations of limonene and ozone were 900/80 μg/m^3 (162/41 ppb) and 80/10 μg/m^3 (14/5 ppb), respectively. The mixture was composed of gaseous products and SOA (mean 80 μg/m^3) (Hagerman et al., 2014). Thus, the initial and residual concentrations of limonene and ozone were substantially higher than commonly found in indoor air, but far below those causing sensory irritation or lung reactions (Wolkoff et al., 2012; Wolkoff and Nielsen, 2017). However, the residual limonene concentration was twice its P_{50} odor threshold (Cain et al., 2007); thus, the odor perception of limonene and its reaction products were intense and possibly unpleasant to some of the subjects. This may have influenced the parasympathetic tone, in agreement with Glass et al. (2014); however, the SOA may also have been causative.

In the sixth study, subjects (n = 30), generally healthy except two with moderate allergic symptoms, were exposed double-blind for 2 h to mixtures of VOCs emitted from either spruce (control) or pine timber (test) wooden planks. These were built into a climate chamber (7.2 m^3, 0.5 h^{-1}), which was ventilated with filtered outdoor air with a median ozone concentration about 13 ppb; the exposure was a randomized cross-over study. The sum of mono-terpene concentrations and (total VOC), was dominated by α-pinene and 3-carene, were in the control and test conditions 1 (35) and 172 (288) ppb, respectively. Subjective and objective measures for the test condition revealed no statistical effects for sensory irritation in eyes (blink frequency), lung function (FEV_1), and inflammation in the airways (FeNO). Furthermore, the exposures showed no impact on two psychological behavior tests, like reaction time and color-word testing (Skulberg et al., 2019).

In summary, the exposure of subjects to near realistic exposure of ozone-terpene mixtures for about two to 4 h in climate chambers only indicated very weak sensory irritation in the eyes or not observed. Further, inflammation was observed neither in nasal lavage nor in exhaled air. The short-term eye-alone exposure studies indicate that high-

end concentrations of ozone and limonene may induce sensory irritation in the eyes, which may be exacerbated at extended exposure time, elderly subjects, and low indoor air humidity conditions in accordance with Wolkoff (2018).

2.7. Human reference values

Human reference values for life-long exposure have been derived from a mouse inhalation model for key oxygenated species such as 4-AMCH, IPOH, 6-methyl-5-heptene-2-one (6-MHO), dihydrocarvone, and 4-OPA. Pulmonary irritation was not observed as a critical effect for these oxidation products; relatively low reference values were derived for airflow limitation (bronchoconstriction) for 4-OPA (123 μg/m^3, 30 ppb) and sensory irritation for IPOH (1100 μg/m^3, 160 ppb) (Wolkoff et al., 2013, 2014, 2016). Although the number of reference values is limited to a few oxidation products, it is important to note that the major effect from α-pinene or limonene reaction mixtures is sensory irritation in the upper airways, mainly by formaldehyde (Wolkoff et al., 2008); further, no sign of an increase upon repeated exposure or increase of effects in the lower airways was observed (Wolkoff et al., 2012). Furthermore, observations did not indicate inflammation in BAL. Thus, it is suggested, using an assessment factor of five that sensory irritation reactions in the eyes and airways would occur by admixing ozone greater than 0.1 ppm with high limonene concentrations.

2.8. Real life exposures

Indoor ozone concentrations are generally between 20 and 60% of outdoor levels, usually between less than 5 ppb and 50 ppb, e.g. Fadeyi (2015). Indoor concentrations of the most common terpenes are generally in the low μg/m^3 range; except for acute activity-related actions resulting in brief peak exposures or continuous use of high-emitting air fresheners (Geiss et al., 2011; Glas et al., 2015; Mandin et al., 2017; Nørgaard et al., 2014a) or simultaneous multi-use of consumer products (Trantallidi et al., 2015). These compounds, in the absence of ozone, are not considered by inhalation indoors to cause sensory irritation effects in the eyes and airways or sensitizing effects in the airways in the general population (Wolkoff and Nielsen, 2017; Basketter et al., 2019; Johnson et al., 2019). However, formaldehyde and hydrogen peroxide can be formed in small amounts in the presence of ozone together with low amounts of gaseous oxidation products and SOA (e.g., Rösch et al., 2017); for instance, by orange peeling (Langer et al., 2008; Vartiainen et al., 2006) and use of household fragrance emitting cleaning products (Wainman et al., 2000; Vartiainen et al., 2006; Langer et al., 2008; Nørgaard et al., 2014b). Thus, orange peeling in an office room (22 m^3; AER = 2 h^{-1}) showed SOA formation about 50 times larger than using a limonene (0.01% undiluted) floor cleaning agent at about 10 ppb ozone (Langer et al., 2008).

Measurements of formaldehyde and 4-OPA in a simulated user scenario under controlled conditions with a plug-in air freshener resulted in maximum levels (background adjusted) reaching 50% and 32%, respectively, of their human reference values (Nørgaard et al., 2014b). Two-hour average levels of formaldehyde, 4-AMCH, IPOH, and 4-OPA in European offices reached combined maximum hazard index levels not greater than 25% and 21%, respectively, for sensory irritation and airflow limitation (Nørgaard et al., 2014a), and with levels similar to predicted in the model study by Carslaw (2013). Further, deep cleaning of all surfaces in one office with textile carpeting resulted in substantial reduction of 4-OPA, which indicates removal of human debris (dirt), such as skin oils and skin cells, as a major ozone-reactive precursor source, e.g. unsaturated fatty acids and squalene, cf. Wisthaler and Weschler (2010), Xiong et al. (2019).

In line with the classic modeling study of the hydroxyl radical from ozone-initiated reactions, Carslaw et al. (2017) measured the hydroxyl and hydroperoxyl radicals in a large office following surface cleaning of desks with a terpene fragranced cleaner. The radical concentrations increased during the cleaning and increased further a factor 2–3 during operation of an air cleaner device to levels high enough to initiate reactions with less reactive VOCs, e.g. aromatics.

Downstream 4-OPA up to 41 μg/m^3 (10 ppb) has been measured from used ozone exposed ventilation filters (Destaillats et al., 2011) and concentrations from 8 up to 39 μg/m^3 have been measured in aircraft cabin and office air (Weschler et al., 2007; Wisthaler and Weschler, 2010); thus, leading to a tentative mean background level of 10 μg/m^3 in offices, possibly from oxidation of squalene. Indoor concentrations of 6-MHO have been measured from 0.8 ppb in offices (Salonen et al., 2009) to 2.3 ppb in a simulated office (28.5 m^3; AER = 1 h^{-1}) with two subjects and an initial ozone concentration of 33 ppb (Wisthaler and Weschler, 2010). Concentrations were 3–6 ppb in an occupied simulated aircraft cabin exposed to ozone (60–70 ppb; AER = 4.4–8.8 h^{-1}) (Weschler et al., 2007) and a mean value of 2 ppb was measured in 14 aircraft cabins in China (Wang et al., 2014a). Wolkoff et al. (2016) reviewed measurements in offices and aircraft cabins and assessed formaldehyde and acrolein to be the major contributors of sensory irritants.

Nørgaard et al. (2014a) measured very low 2-h mean concentrations of 3-AMCH, IPOH, and 4-OPA, and formaldehyde in four European offices. Occupant-dependent maximum levels of 6-MHO and 4-OPA in classrooms were measured to about 0.6 and 0.5 ppb, respectively (Xiong et al., 2019).

In summary, measurements of some of the key oxidation products, acids, formaldehyde, 4-AMCH, IPOH, 6-MHO, and 4-OPA, have been measured in offices, classrooms, aircraft cabins, or modeled at low ppb levels, highest in aircraft cabins, see Table 1.

3. Risk assessment

Established guideline or derived reference values for sensory irritation in the eyes and airways for the most common reaction products, e.g. formaldehyde (Nielsen et al., 2013), 6-MHO and 4-OPA (Wolkoff et al., 2013, 2014), are at least one order of magnitude higher than reported concentrations in office-like environments to be of concern, see Nørgaard et al. (2014a) and Wolkoff et al. (2016). Exception may be an user-related case with a high background of these reaction products originated from ozone-initiated surface reactions on strongly contaminated surfaces, thus adding to the concentration of gas-phase reaction products, cf. Nørgaard et al. (2014b); such a case, however, would require a long-term constant emission. In most cases, the fragrance exposure will occur temporarily with acute high concentrations; exceptions could be excessive use of air fresheners and essential oils. It was previously assessed that ozone concentrations greater than 0.1 ppm may be of concern at high limonene levels (Wolkoff et al., 2012), cf. Klenø and Wolkoff (2004), however, relevant to note that people with susceptible eyes perceive sensory irritation at lower levels, especially at low indoor air humidity conditions (Wolkoff, 2018).

Knowledge about the airway toxicology of ozone-initiated terpene generated SOA ultrafine particles is limited to acute airway effects. Thus, in a mice exposure study the denuded ultrafine SOA in an ozone-limonene mixture did cause neither acute sensory irritation nor airflow limitation (Wolkoff et al., 2008). Furthermore, *in-vitro* and *in-vivo* studies with human lung cells and rodents have not convincingly shown ozone-initiated terpene SOA to cause inflammation at indoor relevant levels, for discussion, see above.

An integrated assessment of ozone-initiated terpene (fragrance) reactions, in the end, should also include potential beneficial effects, not only of terpene-fragrances themselves (Quintans et al., 2018; Johnson et al., 2019), but possibly also of their reaction products, see Wolkoff and Nielsen (2017).

4. Conclusion

Based on this overview, it can be concluded that:

- Human exposure studies to indoor realistic ozone/limonene/terpene mixtures for 2–4 h do not indicate significant inflammatory or (acute) sensory effects in the eyes and airways or pulmonary effects; however, with the exception that weak sensory irritation may occur in the eyes at high-end concentrations and possibly exacerbated at low indoor air humidity conditions.
- Field measurements and modeling studies show levels of "key" oxidation products far below values for (acute) sensory irritation in the eyes and upper airways and reference values for airflow limitation and pulmonary irritation. On the other hand, chamber studies, that simulate user scenarios with only one consumer product, indicate that oxidation products could reach levels of concern in case of simultaneous multi-use of terpene-rich consumer products and excessive indoor ozone. This, however, will strongly depend on indoor surfaces and AER.
- Limonene-ozone reaction mixture appears to possess anti-inflammatory properties as demonstrated in sensitized rodents.
- The health role of produced SOA is less clear. Assessment of levels with outdoor air quality guidelines (e.g. WHO) should not be carried out, because of substantial differences in their morphology and organic composition from ambient (traffic/combustion) particles. There is no direct support for acute effects or inflammation in the upper airways. However, it is fair to speculate whether chronic exposure to SOA may reveal long-term adverse effects; thus, there is a need for long-term testing of effects, like cardiovascular effects.
- An integrated health assessment should apart from an assessment that is based on existing human reference values and indoor air quality guidelines, also include potential beneficial effects of ozone-initiated reactions, like anti-inflammatory properties and sanitary effect of ozone and oxygenated radicals that may alter the building/furniture surfaces and skin surface microbiome.
- Deep cleaning of soiled (carpet) surfaces reduces ozone-initiated surface reactions.
- Clean fleecy surfaces may quench the reactivity of ozone due to high deposition velocity.
- The perceived air quality may be altered by ozone exposure to some treated or soiled surfaces and human skin and debris.

Future actions and questions:

- Can user scenarios be identified with levels of formaldehyde, acrolein, 4-OPA, and SOA sufficiently high causing adverse effects in the eyes, airways, cardiovascular and pulmonary effects by repeated long-term exposure?
- To investigate and quantify anti-inflammatory effects of terpenes and their ozone-initiated terpene reaction mixtures.
- To develop biological cell-based models that mimic realistic exposure scenarios in parallel with/and validated against *in vivo* inhalation models, and human exposure data.
- The witch-hunting of terpene-fragrances and ozone-initiated terpene-fragrance chemistry advocating for banning of fragrances should be moderated in accordance with state-of-the-science toxicology of the terpenes (Wolkoff and Nielsen, 2017; Basketter et al., 2019; Johnson et al., 2019) and combined with present risk assessment of common reaction products, and jointly with an integrated assessment, which incorporates possible beneficial psychological and physiological effects of fragrance terpenes and their zone chemistry.

Declaration of competing interest

The author declares no conflicts of financial interest.

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Phthalate metabolites in urine of children and adolescents in Germany. Human biomonitoring results of the German Environmental Survey GerES V, 2014–2017

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ABSTRACT

During the population representative German Environmental Survey of Children and Adolescents (GerES V, 2014–2017) 2256 first-morning void urine samples from 3 to 17 years old children and adolescents were analysed for 21 metabolites of 11 different phthalates (di-methyl phthalate (DMP), di-ethyl phthalate (DEP), butylbenzyl phthalate (BBzP), di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DnBP), di-cyclohexyl phthalate (DCHP), di-n-pentyl phthalate (DnPeP), di-(2-ethylhexyl) phthalate (DEHP), di-iso-nonyl phthalate (DiNP), di-iso-decyl phthalate (DiDP) and di-n-octyl phthalate (DnOP)).

Metabolites of DMP, DEP, BBzP, DiBP, DnBP, DEHP, DiNP and DiDP were found in 97%–100% of the participants, DCHP and DnPeP in 6%, and DnOP in none of the urine samples. Geometric means (GM) were highest for metabolites of DiBP (MiBP: 26.1 µg/L), DEP (MEP: 25.8 µg/L), DnBP (MnBP: 20.9 µg/L), and DEHP (cx-MEPP: 11.9 µg/L). For all phthalates but DEP, GMs were consistently higher in the 3–5 years old children than in the 14–17 years old adolescents. For DEHP, the age differences were most pronounced. All detectable phthalate biomarker concentrations were positively associated with the levels of the respective phthalate in house dust.

In GerES V we found considerably lower phthalate biomarker levels than in the preceding GerES IV (2003–2006). GMs of biomarker levels in GerES V were only 18% (BBzP), 23% (MnBP), 23% (DEHP), 29% (MiBP) and 57% (DiNP) of those measured a decade earlier in GerES IV.

However, some children and adolescents still exceeded health-based guidance values in the current GerES V. 0.38% of the participants had levels of DnBP, 0.08% levels of DEHP and 0.007% levels of DiNP which were higher than the respective health-based guidance values. Accordingly, for these persons an impact on health cannot be excluded with sufficient certainty.

The ongoing and substantial exposure of vulnerable children and adolescents to many phthalates confirms the need of a continued monitoring of established phthalates, whether regulated or not, as well as of potential substitutes. With this biomonitoring approach we provide a picture of current individual and cumulative exposure developments and body burdens to phthalates, thus providing support for timely and effective chemicals policies and legislation.

1. Introduction

Phthalates (alkyl or aryl esters of phthalic acid) are synthetic organic chemicals with an annual consumption of several million tons worldwide (Micromarket Monitor, 2015). They are used as plasticisers in a variety of industrial applications, as well as in consumer goods and personal care products (Calafat et al., 2015; Koch and Calafat, 2009; Wang et al., 2019). Since phthalates are not chemically bound to the materials to which they are added, they can be found as widespread contaminants in indoor air, house dust and food. Subsequently, humans are primarily exposed to phthalates by ingestion, inhalation and dermal contact (Becker et al., 2009; CDC, 2009; Choi et al., 2017; Den Hond et al., 2015; Heudorf et al., 2007; Koch et al., 2017; Salthammer et al., 2018; Saravanabhavan et al., 2013).

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Abbreviations

BE	biomonitoring equivalent
CAS	Chemical Abstract Service
CHMS	Canadian Health Measures Survey
CI	confidence intervall
EU	European Union
DEMOCOPHES	Demonstration of a Study to Coordinate and Perform Human Biomonitoring on a European Scale
ESB	German Environmental Specimen Bank
GerES	German Environmental Survey
GerES IV	German Environmental Survey on Children
GerES V	German Environmental Survey on Children and Adolescents 2014–2017
GM	geometric mean
G-EQUAS	German External Quality Assessment Scheme
HBM	human biomonitoring
HBM-I-value	human biomonitoring value I
HBM4EU	European Human Biomonitoring Initiative
KiGGS Wave 2	German Health Interview and Examination Survey for Children and Adolescents, Wave 2
KoNEHS	Korean National Environmental Health Survey
LOQ	limit of quantification
m	months
N	sample size
NC	not calculated
NHANES	United States National Health and Nutrition Examination Survey
P	percentile
PVC	polyvinyl chloride
REACH	European chemicals legislation concerning the registration, evaluation, authorisation and restriction of chemicals
RfD	reference dose
RKI	Robert Koch-Institute, Germany
SES	socioeconomic status
SVHC	substance of very high concern
TDI	tolerable daily intake
UBA	German Environment Agency
USEPA	United States Environmental Protection Agency
y	years

Several phthalates have shown a variety of adverse health effects in humans and in animals (Koch and Calafat, 2009; Mariana et al., 2016), of which the most prominent are the endocrine disrupting and reprotoxic effects (summarised by Benjamin et al., 2017; Heudorf et al., 2007; Koch et al., 2017; Lioy et al., 2015; Radke et al., 2018). In addition, results of epidemiological studies also suggest associations between phthalate exposure and overweight, insulin resistance, asthma, attention deficit and attention deficit hyperactivity disorder (Engel et al., 2010; Franken et al., 2017; Hatch et al., 2010; Wang et al., 2015).

Because of their reproductive toxicity butylbenzyl phthalate (BBzP), di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DnBP), di-(2-ethylhexyl) phthalate (DEHP), di-cyclohexyl phthalate (DCHP), and di-n-pentyl phthalate (DnPeP) were classified as substances of very high concern (SVHC) and therefore included in the candidate list of SVHC for authorisation under the European chemical regulation REACH (registration, evaluation, authorisation and restriction of chemicals) (Annex XIV, EC, 1907/2006) (EU, 2006). DEHP, BBzP, DiBP, DnBP and in future DnPeP must not be used within the European Union (EU) without authorisation. Further restrictions for these substances will be implemented in July 2020 (EU, 2018). In addition, di-iso-nonyl phthalate (DiNP), di-iso-decyl phthalate (DiDP) and di-n-octyl phthalate (DnOP) are also restricted to different degrees in children's toys and childcare articles (Annex XVII EC, 1907/2006) (EU, 2006). Several phthalates are also restricted in cosmetics products (EC/1223/2009) (EU, 2009) and in materials intended to come into contact with food (EC/10/2011) (EU, 2011). Similarly, use restrictions, authorisation obligations, and bans were enacted also by the United States (CPSC, 2014) and Canada (Health Canada, 2016).

First human biomonitoring (HBM) studies on phthalates revealed that the general population is ubiquitously and simultaneously exposed to several phthalates (Blount et al., 2000; Koch et al., 2003b; Silva et al., 2004). Since then, phthalates are routinely determined in many HBM studies and national HBM programmes for example in the United States National Health and Nutrition Examination Survey (NHANES) (Calafat, 2012; CDC, 2019), the Canadian Health Measures Survey (CHMS) (Haines et al., 2017), the Korean National Environmental Health Survey (KoNEHS) (Choi et al., 2017), the German Environment Survey (GerES) (Kolossa-Gehring et al., 2012b), and the European DEMOCOPHES Study (Demonstration of a Study to Coordinate and Perform Human Biomonitoring on an European Scale) (Den Hond et al., 2015). The DEMOCOPHES succeeding European Human Biomonitoring Initiative HBM4EU (www.hbm4eu.eu), which is coordinating human biomonitoring in Europe in order to support policy making (Ganzleben et al., 2017), identified phthalates as substances of priority interest for which various policy relevant questions have to be answered by tailored research.

In Germany, urinary phthalate measurements have been carried out in local studies (Kasper-Sonnenberg et al., 2012, 2014; Koch et al., 2011) as well as in the German Environmental Survey on Children, GerES IV (Becker et al., 2004, 2009; Koch et al., 2007a; Schulz et al., 2012; Wittassek et al., 2007) and the German Environmental Specimen Bank (ESB) (Koch et al., 2017; Kolossa-Gehring et al., 2012a).

GerES is part of a health-related environmental surveillance system in Germany (Kolossa-Gehring et al., 2012a, 2012b). The main instruments of GerES are HBM, ambient monitoring of drinking water, house dust, indoor air, noise, and the collection of information on exposure via questionnaires. The target population of GerES V (German Environmental Survey on Children and Adolescents), carried out between 2014 and 2017, were participants aged 3–17 years (Schulz et al., 2017).

In the present paper we describe the urinary levels of 21 phthalate metabolites of 11 parent phthalates in children and adolescents in Germany in a population representative sample, and the associations with some potential predictors of exposure. We compare the results with those of the preceding GerES IV. Our data are used as a basis to calculate and update reference values for these chemicals in Germany. The results also contribute to the overarching goal of HBM4EU to gain current HBM data on the exposure of the European population to chemicals of concern in order to enhance chemical safety.

2. Material and methods

2.1. Study population and sample collection

From January 2015 to June 2017, a population representative sample was recruited in GerES V, which was conducted in close cooperation with the German Health Interview and Examination Survey for Children and Adolescents (KiGGS Wave 2) of the Robert Koch-Institute (RKI) (Mauz et al., 2017). RKI recruited a population representative sample in 167 sampling locations in Germany (Kamtsiuris et al., 2007; Kurth et al., 2008). Out of these, the 3–17 years old GerES V participants were randomly selected as a subsample in the course of the KiGGS Wave 2 examination (Mauz et al., 2017).

In GerES V various environmental contaminants were measured in blood, morning urine, tap water, indoor air and house dust samples. Additionally, questionnaires were used to obtain information on exposure relevant conditions, habits, and behaviors of the participants

(Schulz et al., 2017).

A visit at the homes of the participants was the essential component of the GerES V fieldwork. Kantar Health Munich conducted the fieldwork on behalf of the German Environment Agency (UBA). During the home visits, the fieldworkers inter alia received first void urine, collected dust bags in a subsample of the participants, and conducted interviews either with the participants or their parents.

The first void urine samples were taken either in polypropylene vessels or in narrow-necked polyethylene containers, depending on sex and age of the participant. The samples were kept cold, aliquoted in polypropylene tubes, frozen at the same day, and kept frozen (−20 °C) until analysis. None of the pretested containers had detectable levels of the investigated phthalate metabolites. Samples were analysed in a randomised sequence to avoid observer bias.

The project was approved by the Ethics Committee of the Berlin Chamber of Physicians (Eth-14/14) and the Federal Officer for Data Protection and Freedom of Information (III-425/009#0018).

2.2. Chemical analysis

Analysis of phthalate metabolites in urine was performed by the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance at the Ruhr-University Bochum, Germany. It was executed by on-line high performance liquid chromatography coupled to tandem mass spectrometry, using internal isotope-labelled standards according to previously published methods (Koch et al., 2003a, 2007b, 2012, 2017; Preuss et al., 2005). Creatinine of the urine samples was quantified by the Analytisch-Biologisches Forschungslabor München, Germany, using the Jaffé method (Blaszkiewicz and Liesenhoff-Henze, 2010).

Internal quality control measures for phthalate metabolites were performed throughout the entire period by analysing control urines with known concentrations. These quality control samples were always measured within the ± 3σ range. Additionally, blinded repeated measurements of samples in different analytical cycles always resulted in concentrations within the range of the respective confidence intervals and no metabolite was ever determined in field blanks. The quality of determined creatinine was confirmed by similar internal quality control measures. External quality assurance was confirmed within regular, biannual participation in ring trial program of the German External Quality Assessment Scheme (G-EQUAS) for creatinine, for the metabolites of DEHP, as well as for MnBP, MiBP and MBzP. For the other analytes no external quality assurance was offered. The limits of quantification (LOQ), and the Chemical Abstract Service (CAS) numbers of the measured phthalate metabolites are listed in Table 1.

For phthalate analyses in house dust, the 63 µm dust fraction was analysed with gas or liquid chromatography/mass spectrometry based on Nagorka et al. (2011). The Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany seived and extracted the dust samples either with toluene for gas chromatography or with acetonitrile for liquid chromatography. Quality controls were carried out with inter-laboratory comparisons and constant cross-control measurements of DEHP with gas and liquid chromatography.

2.3. Statistical analysis

In order to adjust the collected sample of GerES V with data from the official demographic statistics of the German population from 2013 to 2015 (Microzensus, 2019), weighting variables based on the key variables age, sex, community size and region were calculated by the RKI (Hoffmann et al., 2018). Subsequently, weighted samples were used in all statistical evaluations whereby the sample and subsample characteristics were calculated from the respective case weighted samples.

Characteristics of the urinary phthalate metabolite distributions were calculated (sample size (N), percentage above the LOQ of the respective phthalate metabolite as listed in Table 1 (% > LOQ), geometric mean (GM), confidence intervals (CI) and percentiles (P)). Additionally, weight sums of metabolites were built for the individual phthalates, e.g. Σ(MiBP + OH-MiBP) for DiBP and Σ(MEHP + OH-MEHP + oxo-MEHP + cx-MEPP) for DEHP. Volume-based as well as creatinine-adjusted concentrations were presented. Concentrations below the LOQ of the respective analytical method were assigned a value equal to half of the LOQ for calculation purposes. Due to the skewed (approximately log-normal) distribution of the metabolite concentrations, GM is a parameter more suitable for assessment than the arithmetic mean.

In the basic evaluation the urinary biomarker levels were described for the total sample as well as for the standard stratification variables: sex, age group, community size, socioeconomic status, region of residence in former East or West Germany, and migration background. In addition, urinary levels for subgroups of substance-specific variables were also described, which are suspected either by scientific knowledge or by biological plausibility to be associated with the metabolite

Table 1
Phthalates measured in GerES V. Parent substances, CAS numbers, metabolites measured, and the respective limits of quantification (LOQ).

Phthalate	Name of parent substance	CAS Number	Metabolite	Name of metabolite	LOQ (µg/L)
DMP	**Di-methyl phthalate**	131-11-3	MMP	Mono-methyl phthalate	1.0
DEP	**Di-ethyl phthalate**	84-66-2	MEP	Mono-ethyl phthalate	0.5
BBzP	**Butylbenzyl phthalate**	85-68-7	MBzP	Mono-benzyl phthalate	0.2
DiBP	**Di-iso-butyl phthalate**	84-69-5	MiBP	Mono-iso-butyl phthalate	1.0
			OH-MiBP	Mono-hydroxy-iso-butyl phthalate	0.25
DnBP	**Di-n-butyl phthalate**	84-74-2	MnBP	Mono-n-butyl phthalate	1.0
			OH–MnBP	Mono-hydroxy-n-butyl phthalate	0.25
DCHP	**Di-cyclohexyl phthalate**	84-61-7	MCHP	Mono-cyclohexyl phthalate	0.2
DnPeP	**Di-n-pentyl phthalate**	131-18-0	MnPeP	Mono-n-pentyl phthalate	0.2
DEHP	**Di-(2-ethylhexyl) phthalate**	117-81-7	MEHP	Mono(2-ethylhexyl) phthalate	0.5
			OH-MEHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate	0.2
			oxo-MEHP	Mono(2-ethyl-5-oxohexyl) phthalate	0.2
			cx-MEPP	Mono(2-ethyl-5-carboxypentyl) phthalate	0.2
DiNP	**Di-iso-nonyl phthalate**	28553-12-0;	OH-MiNP	Mono(4-methyl-7-hydroxyoctyl) phthalate	0.2
		68515-48-0	oxo-MiNP	Mono(4-methyl-7-oxooctyl) phthalate	0.2
			cx-MiNP	Mono(4-methyl-7-carboxyheptyl) phthalate	0.2
DiDP	**Di-iso-decyl phthalate**	26761-40-0;	OH-MiDP	Mono-hydroxy-isodecyl phthalate	0.2
		68515-49-1	oxo-MiDP	Mono-oxo-iso-decyl phthalate	0.2
			cx-MiDP	Mono(2,7-methyl-7carboxy-heptyl) phthalate	0.2
DnOP	**Di-n-octyl phthalate**	117-84-0	MnOP	Mono-n-octyl phthalate	0.2
Various			MCPP[a]	Mono(3-carboxypropyl) phthalate	0.5

[a] Metabolite of several phthalates (currently known: DnBP, DnPeP, DnOP, DiNP, DiDP).

concentrations: carpets with or without plastic backing or underlay, polyvinylchloride (PVC) flooring, wearing of plastic or rubber shoes without socks, habit of chewing on plastic objects, consumption of fast food or ready meals before urine sampling, and concentration of the specific phthalate in the house dust. Excepting phthalate concentration in house dust, all stratification variables were collected by questionnaires. Phthalate levels in house dust were chemically quantified. Bivariate statistical analyses were performed for each variable selected for stratification and when at least 50% of the measured concentrations were equal or above LOQ. Thereby differences of the GM of the subgroups were tested for significance by one-way ANOVA, based on log-transformed data. Significance levels of $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***) were marked for any differences within the categories of the stratification variables.

No significance tests were applied for MCHP, MnPeP and MnOP as they were only detected in very low frequency.

All statistical analyses were performed with the SPSS statistical package (versions 20 and 25).

3. Results and discussion

In GerES V, 2294 children and adolescents participated with complete data, amounting to 75.7% of the eligible persons. From those, 2256 provided sufficient urine volume to determine phthalate metabolite concentrations. Due to chromatographic interferences, some phthalate metabolites could not be determined quantitatively in all samples, resulting in 2247–2256 datasets available for individual phthalate biomarkers. The characteristics of the weighted study population as well as the distribution of several variables, which might be associated with the exposure to phthalates, are shown in Table 2. As the study population was adjusted for the key variables age, sex, community size and region by weighting variables, it can be concluded that the phthalate metabolites were determined in samples representing the 3–17 years old population in Germany.

3.1. Urinary concentrations

Table 3 summarises descriptive statistics for urinary levels of the 21 phthalate metabolites measured. We found metabolites of DMP, DEP, BBzP, DiBP, DnBP, DEHP, DiNP and DiDP at levels > LOQ in 97–100% of the participants' urine samples. In contrast, only 6% of the participants had DCHP and DnPeP and none had DnOP biomarker concentrations > LOQ. The simultaneous detection of DEP, BBzP, DiBP, DnBP, DEHP, DiNP metabolites in almost all urine samples are in line with the results in GerES IV, ESB, NHANES, CHMS, KoNEHS and DEMOCOPHES, where metabolites of these phthalates, when analysed, were also found in almost all urine samples (Becker et al., 2009; Choi et al., 2017; Den Hond et al., 2015; Haines et al., 2017; Koch et al., 2017; Zota et al., 2014). The only exceptions were DMP and DiNP, which were detected in CHMS (2007–2011) less frequently (DMP) or even not at all (DiNP) (Haines et al., 2017). However, this may be due to the higher limits of detection in CHMS.

The highest urinary metabolite concentration was found for MiBP with a GM of 26.1 µg/L urine, followed by MEP (GM of 25.8 µg/L urine), MnBP (GM of 20.9 µg/L urine) and cx-MEPP (GM of 11.9 µg/L urine). Lower concentrations were found for OH-MiNP (GM of 6.9 µg/L urine), MMP (GM of 6.4 µg/L urine), MBzP (GM of 3.1 µg/L urine), and OH-MiDP (GM of 1.5 µg/L urine). Taking the sums of biomarkers for each phthalate, the ranking order of measurable GMs was DiBP > DEHP > DEP > DnBP > DiNP > DMP > DiDP > BBzP. A direct comparison between urinary metabolite levels in terms of exposure to the respective phthalate however is not possible, as urinary excretion fractions differ considerably between the different phthalates and their metabolites. To extrapolate exposure from urinary concentration, urinary metabolite conversion factors, daily urine volume and other anthropometric factors are necessary (Koch et al., 2017).

Table 2
Characterization of the weighted study population for phthalates in GerES V and frequency of various environmental factors, suspected to be related with phthalate exposure.

	N (%)
Children and adolescent	**2256**
Sex	
boys	1164 (52)
girls	1092 (48)
Age group	
3–5 years	402 (18)
6–10 years	736 (33)
11–13 years	457 (20)
14–17 years	662 (29)
Community size	
< 50,000 inhabitants	593 (26)
50,000 - ≤100,000 inhabitants	143 (6)
≥100,000 inhabitants	1520 (67)
Socio-economic status[a]	
low	465 (21)
medium	1320 (58)
high	405 (18)
Region of residence	
West Germany (including West Berlin)	1898 (84)
East Germany (including East Berlin)	358 (16)
Migration background[b]	
no migration background	1561 (69)
one-sided migration background[c]	230 (10)
two-sided migration background[d]	416 (18)
Carpets, carpet tiles, rugs [e]	
with plastic underlay	912 (40)
without underlay	1115 (49)
PVC flooring	
yes	588 (26)
no	1665 (74)
Wearing of plastic or rubber shoes without socks in summer	
yes	1119 (50)
no	1137 (50)
Habit of chewing on plastic objects	
yes	570 (25)
no	1684 (75)
Consumption of fast food or convenience food before urine sampling	
1 day before	506 (22)
2 days before	332 (15)
more than 2 days/never before	1400 (62)
Phthalate level in house dust	
categorized as low, medium, high [f]	various [g]

Note: Due to rounding to nearest whole numbers, the sum of stratified sample sizes not always exactly corresponds to the total sample size. Further differences are due to missing values in stratification criteria.

[a] Socioeconomic status was generated from the dimensions education, occupation and income as provided by the parents. Low, middle or high socioeconomic status were classified as the first (low), second to fourth (medium) or fifth (high) quintile of an index, built by the equally weighted subscales of education, occupation and income (Lampert et al., 2018).

[b] Migration background was based on the country of birth of the child or adolescent and the parents and of the parents' nationality.

[c] One-sided migration background: defined as having one parent not born in Germany or without German citizenship.

[d] Two-sided migration background: includes children and adolescents who themselves migrated to Germany and have at least one parent who was not born in Germany. Children and adolescents belong also to this group, when both parents were born in a country other than Germany or when they are non-German nationals (Frank et al., 2018).

[e] Participants who reported to have no carpets at all were filtered (N = 229).

[f] Categories of low, medium and high phthalate levels were chosen to comprise approximately one third of the participants each. The limits for the medium categories were for: DMP: 0.41–0.48 µg/g, DEP: 0.47–0.83 µg/g, BBzP: 1.67–5.10 µg/g, DiBP: 5.7–11.3 µg/g, DnBP: 5.1–10.5 µg/g, DCHP 1.4–2.7 µg/g, DEHP 108–212 µg/g, DiNP: 135–402 µg/g, DiDP: 19.5–41.0 µg/g. Low levels were below, high levels were above these values for the respective phthalate.

[g] Phthalate levels in house dust were determined in a subsample of 639–646 participants. For N for the specific phthalate see Table 4 and Supplementary Tables 1–50.

Table 3
Phthalates measured in GerES V. Frequency of quantification, percentiles, maximum, arithmetic mean and geometric mean with 95 %-confidence interval of urinary metabolite levels (in µg/L) of the GerES V participants.

Phthalate	Metabolite	N	% ≥ LOQ	P10	P50	P90	P95	GM	95 %CI GM
DMP	MMP	2256	97	1.9	5.9	23.1	43.2	6.4	6.1–6.7
DEP	MEP	2256	100	7.0	23.1	113	219	25.8	24.6–27.0
BBzP	MBzP	2256	99	0.9	2.9	11.2	18.7	3.1	2.9 - 3.2
DiBP	MiBP	2256	100	9.4	26.2	75.0	110	26.1	25.2–27.0
	OH-MiBP	2256	100	3.1	8.8	26.9	37.5	8.9	8.6–9.3
	Σ MiBP + OH-MiBP			12.7	35.4	100	150	35.3	34.1–36.5
DnBP	MnBP	2256	100	8.3	21.0	53.5	69.6	20.9	20.3–21.6
	OH–MnBP	2256	99	0.8	2.5	6.3	8.5	2.4	2.3 - 2.5
	Σ MnBP + OH–MnBP			9.2	23.4	59.6	77.0	23.4	22.7–24.2
DCHP	MCHP	2256	6	< LOQ	< LOQ	< LOQ	0.3	< LOQ	
DnPeP	MnPeP	2255	6	< LOQ	< LOQ	< LOQ	0.2	< LOQ	
DEHP	MEHP	2256	86	< LOQ	1.5	4.7	6.7	1.4	1.4 - 1.5
	OH-MEHP	2256	100	4.3	11.1	29.1	40.9	11.0	10.6–11.4
	oxo-MEHP	2253	100	2.7	7.7	21.5	29.0	7.6	7.3–7.8
	cx-MEPP	2256	100	4.4	12.0	34.0	46.1	11.9	11.5–12.3
	Σ OH- + oxo-MEHP	2253		7.1	18.8	49.0	70.2	18.6	18.0–19.3
	Σ MEHP + OH-MEHP + oxo-MEHP + cx-MEPP			12.4	32.4	86.9	123	32.5	31.5–33.6
DiNP	OH-MiNP	2249	100	2.4	6.9	19.7	30.2	6.9	6.7–7.2
	oxo-MiNP	2256	99	0.9	2.7	8.6	14.2	2.8	2.7 - 2.9
	cx-MiNP	2250	100	1.9	5.4	19.0	30.2	5.9	5.6–6.1
	Σ OH-MiNP + oxo-MiNP + cx-MiNP			5.5	15.7	47.1	71.9	16.0	15.4–16.6
DiDP	OH-MiDP	2256	98	0.5	1.5	4.9	7.5	1.5	1.5 - 1.6
	oxo-MiDP	2256	88	< LOQ	0.7	2.3	3.6	0.6	0.6 - 0.7
	cx-MiDP	2256	97	0.3	0.9	2.6	4.2	0.9	0.9 - 0.9
	Σ OH-MiDP + oxo-MiDP + cx-MiDP			1.0	3.1	9.6	16.0	3.2	3.1–3.3
DnOP	MnOP	2256	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	
Various	MCPP	2256	92	0.5	1.4	4.1	6.4	1.5	1.4 - 1.5

Abbreviations: N: sample size, LOQ: limit of quantification, P10, P50, P90. P95: percentiles, GM: geometric mean, 95% CI GM: 95% confidence interval for GM. Values below LOQ were set LOQ/2 for calculation purposes. No 95% CI GM is given if GM < LOQ.

The urinary concentrations of phthalate metabolites were also evaluated for the subgroups illustrated in Table 2. In Table 4 we exemplarily show the distributions and statistical parameters for the phthalate metabolite MiBP. The tables for all other metabolites and metabolite sums are compiled in Supplementary Tables 1–26 in µg/L urine and in Supplementary Tables 27–50 in µg/g creatinine.

The most striking result of the bivariate analyses was the age dependence of urinary phthalate metabolite concentration. The GMs of MBzP and the sums of DiBP, DnBP, DEHP DiNP and DiDP urinary metabolite levels, constantly decreased with age, being highest for the 3–5 years old and lowest for the 14–17 years old participants. MEP showed an opposite age effect, the GMs of MEP levels increased with age. As MCHP, MnPeP and MnOP were only detected sporadically, no subgroup differences became apparent. The GMs of phthalate urine levels of 3–5 years old children and 14–17 years old adolescents are illustrated in Fig. 1. Excepting DEP (i.e. MEP), the proportions of GMs of the 3–5 compared to the 14–17 years old participants varied between about 1.3-fold for DMP to about 1.9-fold for DEHP.

No consistent picture can be drawn from the additional socioeconomic and geographic variables suspected to be associated with phthalate exposure. Sex differences were found for some phthalate metabolites, mainly when comparing creatinine-adjusted levels (Supplementary Tables 27–50). Whereas boys had a higher GM for MMP, higher GMs for girls were found for MEP, MnBP, DiNP and DiDP metabolites. Socioeconomic status (SES) was associated with MMP, MEP, MBzP as well as with DiBP, DEHP, and DiNP metabolite levels in urine (see Supplementary Tables 1-3, 5, 16, 20). Whereas GMs of MMP increased with increasing SES, GMs of MEP, MBzP, DiBP, DEHP, and DiNP metabolites were conversely associated and declined with increasing SES. Migration background was associated with higher GMs of MEP, DEHP, and DiNP metabolite concentrations (Supplementary Tables 2, 16, 20). For MBzP GMs of urinary levels also differed with community size (Supplementary Table 3), being smaller in larger communities. Participants living in former East Germany had higher GMs of MMP, DiBP, and DnBP metabolite levels than participants living in former West Germany (Supplementary Tables 1, 5, 8).

When considering variables of living environment and habits, high phthalate concentrations in house dust were associated with the respective phthalate metabolite levels in urine (see Fig. 2 and Supplementary Tables 1-3, 5, 8, 16, 20, 24). With high phthalate concentration in house dust, the participants had 1.6- to 2.0-fold higher GMs of urinary MMP, MEP, MBzP, and DiBP metabolites and 1.2- to 1.3-fold higher GMs of urinary DEHP, DiNP, and DiDP metabolites than with low concentrations of the respective phthalate in house dust. Moreover, PVC flooring and carpets with plastic underlay were mostly positively associated with phthalate concentrations, namely with MMP, MBzP, DnBP, DEHP, and DiNP metabolites, either volume- or creatinine-adjusted or both (Supplementary Tables 1, 3, 8, 16, 20, 27, 29, 35, 41, 45). Levels of DiDP metabolites were only associated with plastic carpet underlays, but not with PVC flooring (see Supplementary Tables 24 and 49). MEP levels were not associated with PVC flooring, which is probably due to its main use in personal care products. Surprisingly, MEP levels were positively associated with plastic underlays (Supplementary Tables 2 and 28). For many phthalate concentrations, positive associations were also found with the habit of chewing on plastic objects, the consumption of fast or convenience food, and wearing of plastic or rubber shoes. These associations, however, were not coherent and could not be substantiated when adjusted for age.

The age dependency of urinary phthalate metabolite levels as revealed in GerES V can be found in many studies (for example Becker

Table 4
Urinary levels of MiBP in subpopulations of the GerES V participants in µg/L urine.

	N	% > LOQ	P10	P50	P90	P95	GM	95% CI GM
Total	2256	100	9.4	26.2	75.0	110	26.1	25.2–27.0
Sex								
boys	1164	100	9.5	26.2	83.6	115	26.3	25.0–27.6
girls	1092	100	9.1	25.9	72.9	104	25.9	24.6–27.1
Age group***								
3–5 years	402	100	10.9	29.5	93.7	143	30.4	27.9–33.2
6–10 years	736	100	10.8	28.8	72.5	122	28.9	27.3–30.5
11–13 years	457	100	8.9	22.7	74.4	114	23.9	22.1–25.8
14–17 years	662	100	8.3	22.2	66.0	88.1	22.5	21.2–24.0
Community size (inhabitants)*								
< 50,000	593	100	9.7	24.5	65.4	100	25.1	23.5–26.7
50,000 - < 100,000	143	100	10.8	31.8	95.1	231	31.4	27.1–36.4
≥100,000	1520	100	9.1	26.2	77.6	110	26.0	24.9–27.1
Socioeconomic status*								
low	465	100	9.5	29.2	75.4	114	28.1	26.1–30.3
medium	1320	100	9.1	25.0	79.3	113	26.0	24.8–27.2
high	405	100	9.7	23.7	62.5	84.7	23.8	22.1–25.6
Region of residence**								
West Germany (including West Berlin)	1898	100	9.1	25.4	74.6	110	25.4	24.5–26.4
East Germany (including East Berlin)	358	100	11.5	29.7	79.0	120	29.6	27.2–32.3
Migration background***								
no migration background	1561	100	9.4	24.9	70.4	100	25.2	24.2–26.3
one-sided migration background	230	100	7.9	27.9	66.2	86.1	25.1	22.7–27.7
two-sided migration background	416	100	11.2	29.9	110	136	30.1	27.5–33.0
Carpets, carpet tiles, rugs								
with plastic underlay	912	100	9.5	27.7	80.6	113	27.0	25.6–28.6
without plastic underlay	1115	100	9.8	26.1	74.6	108	26.3	25.0–27.6
PVC flooring***								
yes	588	100	11.4	29.6	101	141	31.4	29.3–33.7
no	1665	100	9.1	24.4	69.5	95.6	24.4	23.5–25.4
Wearing of plastic or rubber shoes without socks in summer***								
yes	1119	100	8.5	24.9	69.1	107	24.6	23.4–25.8
no	1137	100	9.7	27.0	77.5	113	27.6	26.3–29.0
Habit of chewing on plastic objects*								
yes	570	100	9.7	27.8	82.8	129	28.1	26.2–30.2
no	1684	100	9.4	25.2	71.6	105	25.4	24.4–26.4
Consumption of fast food or convenience food before urine sampling								
1 day before	506	100	9.5	25.6	69.4	127	25.3	23.5–27.2
2 days before	332	100	8.5	28.1	68.2	110	26.7	24.4–29.1
more than 2 days/never before	1400	100	9.4	25.9	78.7	109	26.3	25.2–27.5
House dust levels of DiBP***								
low	188	100	7.1	16.4	43.5	58.5	17.2	15.6–19.1
medium	193	100	12.2	25.9	69.2	105	26.9	24.3–29.8
high	248	100	13.2	32.1	84.5	109	32.4	29.6–35.6

For abbreviations see Table 3. For description of subpopulations see Table 2. Variant sample sizes and sums of sample sizes are due to rounding strategy, filtering and missing values.
Significance test: One-way ANOVA (differences of GM). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. Significance levels mean differences within any of the categories of the respective variable.

et al., 2009; Correia-Sa et al., 2018; Gari et al., 2019; Kasper-Sonnenberg et al., 2014; Wittassek et al., 2007). Likewise, higher phthalate metabolite levels are reported for children when directly compared to adults, the only exception being MEP (CDC, 2019; Den Hond et al., 2015; Schwedler et al., 2017; Zota et al., 2014). This is probably due to a higher food consumption related to body weight of young children, but also to other characteristics such as mouthing habits, or increased dust intake by playing near the ground. The clearly higher burden of young children compared to adolescents and adults and the simultaneous presence of the various phthalate metabolites must be considered when assessing exposure burden.

There are no consistent results for the exposure factors sex and SES in the literature. Only MEP urinary levels were constantly higher in girls than in boys (CDC, 2019; Correia-Sa et al., 2018; Gari et al., 2019; Saravanabhavan et al., 2013). Associations of higher SES with lower urinary levels of MBzP were also found by Gari et al. (2019) in a Polish study, and by Kobrosly et al. (2012) in the NHANES population of 2001–2008. Associations of SES with other phthalate metabolite levels were inconsistent.

House dust levels of the respective phthalate were not associated with the urinary phthalate metabolite concentrations in earlier studies (Becker et al., 2004). However, in more recent studies, school dust (Larsson et al., 2017) and PVC materials at home (Den Hond et al., 2015; Koppen et al., 2019; Schwedler et al., 2017) indeed were associated with urinary phthalate metabolite concentrations, underlining the relevance of the exposure pathways via house dust and indoor air for several phthalates.

A strength of the study is the robust sampling design and the application of sampling weights in the analyses ensuring that the results are representative of the respective population. However, the single urine sample per participant and the cross-sectional study design limits the analyses of associations of urinary phthalate levels and potential predictors of exposure.

3.2. Comparison with results of GerES IV

Phthalates were also measured in GerES IV in 3–14 years old children. This allows comparison of the average urinary phthalate metabolite concentrations between GerES V and GerES IV for this age group (Fig. 3). The GMs of all phthalate metabolite levels measured in both

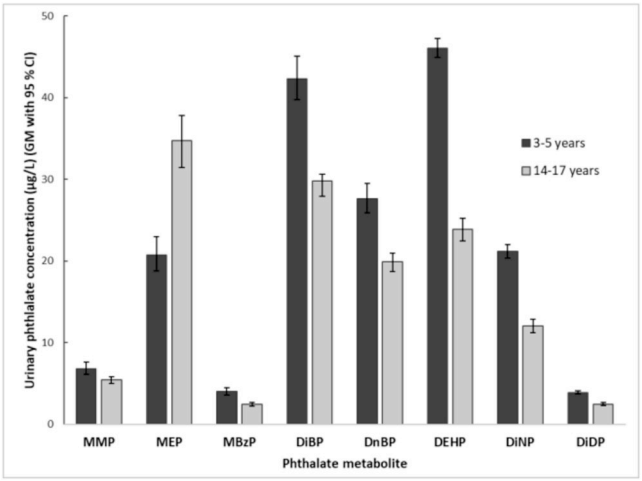

Fig. 1. Urinary phthalate concentrations in younger (3–5 years) and older (14–17 years) GerES V participants.
MMP, MEP and MBzP are the metabolites of DMP, DEP and BzPB, respectively. DIBP, DnBP, DEHP, DiNP, and DiDP are expressed as sums of the following metabolites: DiBP (Σ MiBP + OH-MiBP), DnBP (Σ MnBP + OH–MnBP), DEHP (Σ MEHP + OH-MEHP + oxo-MEHP + cx-MEPP), DiNP (Σ OH-MiNP + oxo-MiNP + cx-MiNP), DiDP (Σ OH-MiDP + oxo-MiDP + cx-MiDP). Details on all age groups are given in the Supplementary Tables 1-3, 5, 8, 16, 20, and 24.

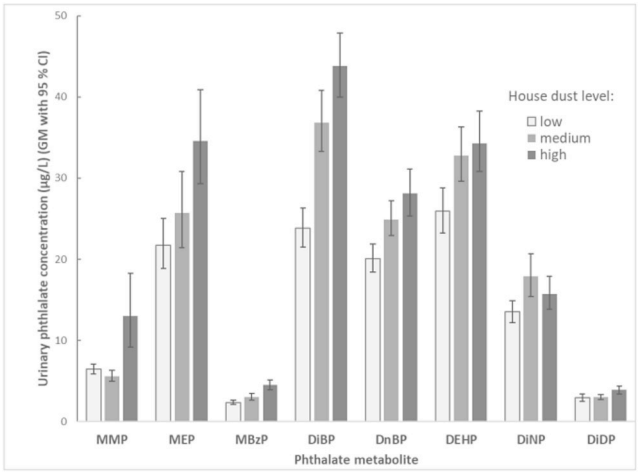

Fig. 2. Urinary phthalate concentrations in association with the respective phthalate levels in house dust.
For the definition of low, medium and high house dust levels see legend to Table 2.
For the description of phthalate and phthalate metabolites see legend to Fig. 1.

surveys were considerably lower in samples collected in the years 2015–2017 (GerES V) than in the years 2003–2006 (GerES IV). The highest difference was observed for MBzP with a GM in GerES V being only 18% of that in GerES IV. GMs of MiBP, MnBP, and DEHP concentrations amounted to only 29%, 23%, and 23% of the GMs found in GerES IV. The lowest difference was found for DiNP with a GM amounting 57% of the GM calculated for GerES IV.

Reduced urinary phthalate concentrations over the last decade were also reported for the German ESB (Koch et al., 2017), NHANES (Calafat et al., 2015; Reyes and Price, 2018b; Zota et al., 2014) and CHMS (Haines et al., 2017). The reduction may be assigned to bans and restrictions in children's toys and childcare articles, in cosmetic products and materials intended to come in contact with food (summarised by Koch et al., 2017), and also to increasing consumer awareness towards these substances (Calafat et al., 2015). The relatively slim reduction of

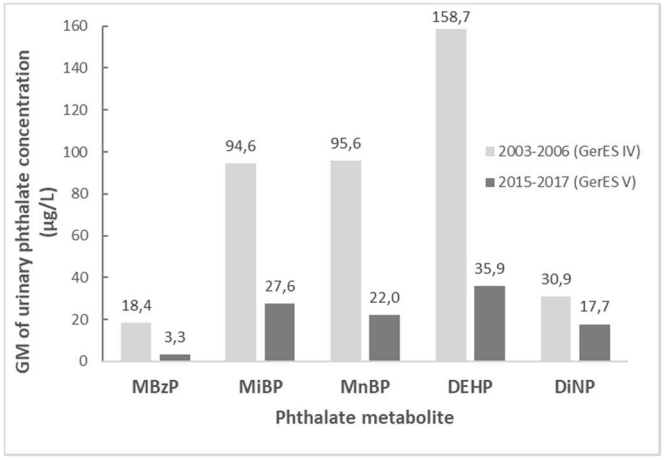

Fig. 3. Comparison of urinary phthalate biomarker levels in 3–14 years old children in Germany. Samples were collected in 2003–2006 (GerES IV) and in 2015–2017 (GerES V).
MBzP, MiBP and MnBP are the metabolites of BBzP, DiBP and DnBP, respectively DEHP and DiNP are expressed as sums of the following metabolites: DEHP (Σ MEHP + OH-MEHP + oxo-MEHP + cx-MEPP), DiNP (Σ OH-MiNP + oxo-MiNP + cx-MiNP).

DiNP, a phthalate introduced in the market as a substitute for DEHP may extend in the future, as it has been restricted just recently. Additionally, current market changes and substituting chemicals must be considered. Newly developed plasticisers like DPHP (di-(2-propylheptyl) phhtalate), DEHTP (di-(2-ethylhexyl) terephthalate), and Hexamoll® DINCH (di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate) were introduced into the market and have seen a constant increase of production volume and applications. Meanwhile results of the German ESB revealed an omnipresent detection of DINCH and DEHTP in urine samples of the German ESB study population (Kasper-Sonnenberg et al., 2019; Lessmann et al., 2019) and a detection of DPHP in one of five ESB participants (Schmidtkunz et al., 2019). DINCH and DEHTP have also been detected in urine samples of the population of the United States in substantial amounts (CDC, 2019; Silva et al., 2013; Silva et al., 2019). DINCH and DPHP measurements in children and adolescents in GerES V revealed that DINCH has reached the bodies of all and DPHP of about 62% of the children and adolescents in Germany at quantifiable levels (Schwedler et al., 2019). The favourable result of reduced phthalate levels in children and adolescents in Germany therefore is contrasted by a clear and extensive exposure to supplementary chemicals. In summary, to evaluate the total body burden with phthalates and plasticisers, not only the reduction of exposure to established phthalates, whether restricted or not, but also the emerging exposure to substitutes must be considered.

3.3. Comparison with other surveys

In Table 5 we compare phthalate metabolite levels determined in GerES V with biomonitoring data of HBM studies of a similar time period and with participants of similar age. Additionally, data of 20–29 years old adults of the German ESB from 2015 (Koch et al., 2017) were included. Compared to the German ESB, the children and adolescents of GerES V throughout had higher median phthalate metabolites levels.

Compared to 6–11 years old children from NHANES (CDC, 2019), 6–10 years old GerES V participants had similar GMs of MEP and DEHP, higher GMs of DnBP, and lower GMs of MBzP, cx-MiNP, and cx-MiDP levels. The same differences were found in GerES IV (Becker et al., 2009) and may reflect country specific production and use patterns.

Similarities and differences were also observed when European studies were compared. MBzP, MiBP, MnBP, MEHP, OH-MEHP, and oxo-MEHP were measured in Czechia (Puklova et al., 2019). The 5 and

Table 5
Comparison of median phthalate levels in urine (μg/L) of GerES V children and adolescents with levels measured in different studies.

Study, region	GerES V[a] Germany	ESB[b] Germany	GerES V[a] Germany	NHANES[c] USA	Czechia[d]	REPRO_PL[e] Poland	Esteban[f] France	GerES V[a] Germany	Sweden[g]	GerES V[a] Germany	Portugal[h]
Year	2015–2017	2015	2015–2017	2015–2016	2016–2017	2014–2015	2014–2016	2015–2017	2015	2015–2017	2014–2015
Age	3–17 y	20–29 y	6–10 y	6–11 y	5 + 9 y	7 y	6–10 y	3–5 y	40–48 m	3–17 y	4–18 y
N	2249–2256	60	727–736	415	370	250		397–402	113	2249–2256	112
	P50	P50	GM	GM	GM	GM	GM	GM	GM	GM	GM
MMP	5.9	2.8	7.3			5.1	5.3	6.8		6.4	3.1
MEP	23.1	13.5	21.7	24.5		42.9	40.6	20.8	32	25.8	58.3
MBzP	2.9	1.2	3.4	10.7	3.65	5.5	9.7	4.0	9.0	3.1	2.25
MiBP	26.2	9.8	28.9	11.2	44.1	76.2	50.1	30.4		26.1	16.8
OH-MiBP	8.8	2.8	10.2	4.04		27.9		11..5		8.9	6.54
MnBP	21	8	22.9	14.4	63	55	27.6	24.2	55	20.9	12.8
OH–MnBP	2.5	0.8	2.7	1.5		7		3.2		2.4	1.67
MCHP	< LOQ	< LOQ	< LOQ			< LOQ	NC	< LOQ		< LOQ	< LOQ
MnPeP	< LOQ	< LOQ	< LOQ			< LOQ		< LOQ		< LOQ	< LOQ
MEHP	1.5	1.1	1.4	1.42	2.31	2.7	2.0	1.4	1.5	1.4	1.9
5OH-MEHP	11.1	4.2	12.7	8.81	20.5	27.1	17.0	15.4	17	11.0	10.9
5oxo-MEHP	7.7	3.2	9.0	5.97	12.8	19.9	12.9	11.0	11	7.6	7.62
5cx-MEPP	12	3.8	14.1	14.6		31.4		17.7	16	11.9	16.1
OH-MiNP	6.9	2.4	8			9.5		9.4	12	6.9	5.57
oxo-MiNP	2.7	0.9	3.1			3.1		3.6	5.9	2.8	2.23
cx-MiNP	5.4	2	6.6	11.1		7.6		7.6	17	5.9	7.42
OH-MiDP	1.5	0.8	1.8			1.8		1.9		1.5	1.31
oxo-MiDP	0.7	0.3	0.7			0.89		0.7		0.6	0.71
cx-MiDP	0.9	0.4	1.1	2.26		0.91		1.2		0.9	1.19
MnOP	< LOQ	< LOQ	< LOQ			< LOQ	NC	< LOQ		< LOQ	< LOQ
MCPP	1.4	0.3	1.8	1.79		2.2		2.1		1.4	1.03

Abbreviations: N: sample size, LOQ: limit of quantification, P50: 50th percentiles, GM: geometric mean, NC: not calculated, y: years, m: months.
[a] This study.
[b] ESB: German Environmental Specimen Bank (Koch et al., 2017).
[c] NHANES: National Health and Nutrition Examination Survey (CDC, 2019).
[d] Czechia (Puklova et al., 2019).
[e] REPRO_PL: Polish Mother and Child Cohort Study (Gari et al., 2019).
[f] Esteban, France (Balicco et al., 2019).
[g] Sweden (Larsson et al., 2017).
[h] Portugal (Correia-Sa et al., 2018).

9 years old Czech children throughout had higher GMs of urinary phthalate metabolite levels than the 6–10 years old participants of GerES V, ranging from 1.1-fold for MBzP to 2.7-fold for MnBP. The GMs of MEP, MBzP, MiBP, MnBP, MEHP, OH-MEHP, and oxo-MEHP, measured in the Esteban 2014–2016 study in France inter alia in 6–10 years old children (Balicco et al., 2019) were also 1.2–2.9-fold higher than those measured in GerES V. Only the GM of MMP was lower in the Esteban than in the GerES V study.

In a Swedish study, children aged 40–48 months were investigated for MEP, MBzP, MnBP, and for DEHP and DiNP metabolites (Larsson et al., 2017). Compared with the 3–5 years old GerES V children, the GMs for urinary DEHP metabolites were similar, whereas the Swedish children had 1.5- to 2.3-fold higher GMs for MEP, MBzP, MnBP, OH-MiNP, oxo-MiNP, and cx-MiNP.

The whole GerES V set of phthalate metabolites was analogously determined in 7 years old children in Poland (Gari et al., 2019) and in 4–18 years old children in Portugal (Correia-Sa et al., 2018). For MCHP, MnPeP, and MnOP, values below LOQ were obtained in all three studies. GMs of urinary DiNP and DiDP metabolite levels were in the same range in the German, Polish, and Portuguese respective age groups (GerES 6–10 years olds compared with Polish 7 years olds, GerES 3–17 years olds compared to Portuguese 4–18 years olds). GMs of the other individual phthalate metabolite levels differed not more than 2.6-fold compared with GerES V data. Polish children had lower GM of MMP and higher MEP, MBzP, DiBP, DnBP, and DEHP levels than the GerES V children. Portuguese children and adolescents had lower GMs of MMP, MBzP, DiDP, and DnBP, higher GMs of MEP, and similar GMs of DEHP levels compared to the German children and adolescents.

In summary, comparison of a similar time period predominantly revealed similarities and only slight differences in GMs of individual phthalate metabolite levels in comparable age groups. As all but one of the compared studies were located in Europe, similar phthalate restrictions and usages may explain the predominant congruence.

3.4. Comparison with health-based guidance values

Health-based guidance values are available for MEP, MBzP, MnBP, for the sum of the 3 DiNP metabolites and for different combinations of DEHP metabolites (Angerer et al., 2011; Apel et al., 2017; Aylward et al., 2009a, b; Hays et al., 2011). The proportions of children and adolescents exceeding either human biomonitoring value I (HBM-I value) derived by the German Human Biomonitoring Commission (Apel et al., 2017) or BE (biomonitoring equivalent) values, derived by Aylward et al. (2009a, 2009b) and Hays et al. (2011) are shown in Table 6. None of the children and adolescents in Germany exceeded BE values for MEP (18000 μg/L urine), BBzP (3800 μg/L urine) and the HBM-I value for DEHP (Σ OH-MEHP and oxo-MEHP) of 500 μg/L for 6–13 years old children.

However, 0.38% of the participants had concentrations of MnBP in urine above the BE value of 200 μg/L urine, exceeding the limit where adverse health effects cannot be excluded with sufficient certainty. Likewise, the BE value of 1800 μg/L for DiNP was exceeded by 0.007%, and for DEHP (260 μg/L for ΣMEHP + OH-MEHP + oxo-MEHP and 400 μg/L for ΣMEHP + OH-MEHP + oxo-MEHP + cx-MEPP) by 0.08% of the participants. Extrapolated to the reference population in Germany, this would represent about 41500, 800, and 9000 children

Table 6
German GerES V participants exceeding health-based guidance values derived for urinary phthalate metabolites.

Phthalate	Biomarkers/metabolites	Source of health based guidance value	Value	% of GerES V participants exceeding health-based guidance value	Extrapolated for the population in Germany aged 3–17 years (↑ 11 million)
DEP	MEP	BE based on USEPA subchronic RfD	18000 µg/L	0	0
BBzP	MBzP	BE based on USEPA subchronic RfD	3800 µg/L	0	0
DnBP	MnBP	BE based on EFSA subchronic TDI	200 µg/L	0.38	~41500
DiNP	OH-MiNP + oxo-MiNP + cx-MiNP	BE based on EFSA subchronic TDI	1800 µg/L	0.007	~800
DEHP	OH-MEHP + oxo-MEHP	HBM-I-value (6-13 years)	500 µg/L	0	0
	MEHP + OH-MEHP + oxo-MEHP	BE based on USEPA subchronic RfD	260 µg/L	0.08	~9000
	MEHP + OH-MEHP + oxo-MEHP + cxMEPP	BE based on USEPA subchronic RfD	400 µg/L	0.08	~9000

Abbreviations: BE: biomonitoring equivalent, EFSA: European Food Safety Authority, GerES V: German Environmental Survey for Children and Adolescents 2014–2017, HBM-I-value: human biomonitoring value I, RfD: reference dose, TDI: tolerable daily intake, USEPA: United States Environmental Protection Agency.
Health based guidance values for DEP, BBzP, DnBP: Aylward et al. (2009a), DiNP: Hays et al. (2011), DEHP: Apel et al. (2017) and Aylward et al. (2009b).

and adolescents, exceeding health-based guidance values for MnBP, DiNP, and DEHP, respectively. These results show that even though regulations of DnBP, DiNP, and DEHP are in force for several years and average phthalate concentrations were lower than in previous studies, a proportion of children and adolescents still exceeds health-based guidance values.

Several phthalates have similar toxicological profiles and there is evidence that they can produce cumulative additive adverse effects (Christiansen et al., 2009; Conley et al., 2018; Furr et al., 2014; Howdeshell et al., 2007, 2017; Reyes and Price, 2018a; Rider et al., 2010). As DnBP and DEHP are among those phthalates suspected to induce comparable endocrine disrupting and reprotoxic effects, exceedances of their health-based guidance values are of special importance.

The ongoing exposure to phthalates, whether regulated or not, confirms the need for continuous monitoring of established as well as of upcoming phthalates and their substitutes. A comprehensive picture of the actual levels and developments of aggregated body burdens and comprehensive health-based guidance values are necessary to support further actions to reduce exposure to plasticisers in the vulnerable group of children and adolescents.

4. Conclusion

The omnipresence of phthalates in daily life is reflected in the body burdens of children and adolescents in Germany. Metabolites of 8 phthalates were found in 97%–100% of the samples. With the exception of MEP, the young children in GerES V were exposed to phthalate metabolites at up to 1.9-fold higher levels than the adolescents. Compared to GerES IV reduced GMs of all measured phthalates were measured in GerES V, which is most probably due to restrictions and regulations in applications and consumer products. However, alternatives and substitutes have entered the market and have to be monitored and evaluated accordingly.

Comparison with other studies for the years 2015–2017 revealed similarities and only slight differences in GMs of individual phthalate metabolite levels. Comparable phthalate restrictions and usages may contribute to these results.

Although regulation, bans, and restrictions are in force for several phthalates and average phthalate concentrations have declined, there are still some children and adolescents with urinary levels exceeding the individual health-based guidance values for DnBP, DEHP, and DiNP.

Maintaining biomonitoring of phthalate metabolites is also necessary to reveal whether the current authorisation of BBzP, DiBP, DnBP and DEHP results in further reduction of urinary levels.

The representative GerES V data on phthalate exposure of children and adolescents will be used to calculate and update reference values for this subpopulation in Germany. Repeated monitoring is necessary to assess the extent of phthalate exposure in the population in the light of their widespread use and to observe the developments due to regulatory restrictions and replacements by substitutes. By providing the best possible exposure data, our results will also contribute to further EU chemicals regulation via the European HBM initiative HBM4EU, which aims to support and promote the protection of all Europeans against environmental health risks.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.113444.

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Prenatal pesticide exposure and respiratory health outcomes in the first year of life: Results from the infants' Environmental Health (ISA) study

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ABSTRACT

Background: Growing evidence suggests that pesticide exposure may influence respiratory health, but data in young children are very limited. We examined the association of prenatal pesticide exposure with lower respiratory tract infections (LRTIs) and wheeze at one year of age in children from the Infants' Environmental Health (ISA) study in Costa Rica.

Methods: We measured seven pesticide metabolites, including ethylenethiourea (ETU, metabolite of mancozeb), in maternal urine samples collected repeatedly during pregnancy. For each woman, we averaged pesticide concentrations during each half of pregnancy ($\leq$20 and $>$ 20 weeks of gestation) and across repeated samples collected over the course of pregnancy. We collected information about LRTIs (n = 355) and wheezing (n = 272) during the first year of life from mothers when their children were 11–19 months old. We fit multivariable logistic regression models using high (quartile 4) vs. low (quartiles 1–3) urinary pesticide concentrations as exposures and adjusted models for maternal age, education, parity, gestational age at birth, and child sex.

Results: Ten percent of the children had at least one LRTI and 39% had at least one episode of wheezing during their first year of life. Median (25-75th percentile) specific gravity-corrected urinary ETU concentrations during the first half, second half, and over the course of pregnancy were 3.4 (2.1–5.0), 3.3 (2.2–4.7), and 3.4 (2.4–5.0) ng/mL, respectively. We observed that high urinary ETU concentrations during the first half of pregnancy were associated with increased odds of LRTI (OR = 2.45; 95% CI: 0.96, 6.26), whereas high urinary ETU concentrations during the second half of pregnancy were associated with decreased odds of wheezing (OR = 0.50; 95% CI: 0.26, 0.96). We found that the association between high urinary ETU concentrations during the first half of pregnancy and LRTIs persisted among mother-child pairs with either high or low ETU concentrations during the second half. In contrast, the association of high urinary ETU concentrations during the second half of pregnancy with wheezing was attenuated when we simultaneously adjusted for urinary ETU concentrations during the first half. We observed null associations between other pesticide metabolites measured during pregnancy and respiratory outcomes.

Conclusions: Our data indicate that exposure to mancozeb/ETU during the first half of pregnancy may be associated with respiratory outcomes in the first year of life.

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; DAP, dialkyl phosphate; DCCA, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyldichloroethane; ETU, ethylenethiourea; ISA, Infants' Environmental Health ('Infantes y Salud Ambiental'); ICC, intraclass correlation coefficient; ISAAC, International Study of Asthma and Allergies in Childhood; LOD, limit of detection; LC-MS/MS, liquid chromatography mass spectrometer; LRTI, lower respiratory tract infection; OH-P, 3-hydroxypyrimetanil; 5-OH-TBZ, 5-hydroxythiabendazol; OP, organophosphate; OR, odds ratio; 3-PBA, 3-phenoxybenzoic acid; P75, 75th percentile; SD, standard deviation; TCPy, 3,5,6-trichloro-2-pyridinol

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1. Introduction

Respiratory outcomes including infection and wheezing during infancy can have long-term consequences for respiratory health (Busse et al., 2010; Jackson et al. 2008, 2016; Jackson, 2014; Lemanske et al., 2005; Liu et al., 2017; Lodge et al., 2014; Rubner et al., 2017). For example, viral wheezing respiratory illnesses during infancy were associated with an increased risk of subsequent wheezing at preschool age (Lemanske et al., 2005) and asthma at school age (Jackson et al., 2008). Similarly, lower respiratory tract infections (LRTIs) in the first year of life have been associated with increased early transient wheeze (before age 3 years) and intermediate-onset wheeze (after age 2 years) in a high risk for allergy birth cohort (Lodge et al., 2014). To date, limited information is available for environmental factors that contribute to LRTIs or wheezing during the first year of life (Bloomberg, 2011; Cupul-Uicab et al., 2014; Dick et al., 2014; Gascon et al. 2012, 2013, 2014; Hehua et al., 2017; McEvoy and Spindel, 2017; Selby et al., 2018; Vanker et al., 2017).

Pesticides have been associated with adverse respiratory outcomes in young children, but the evidence is scarce, as the timing of exposure, pesticides assessed, and outcomes measured have varied among studies. Most analyses have focused on dichlorodiphenyldichloroethylene (DDE), the metabolite of dichlorodiphenyldichloroethane (DDT), an organochlorine pesticide that is both biologically and environmentally persistent. A meta-analysis of 10 European birth cohorts found that higher prenatal DDE concentrations were associated with an increased risk of bronchitis and wheezing in the first 18 months of life, though the magnitude of the observed association was small (Gascon et al., 2014). Prenatal exposure to DDE was also associated with an increased risk of LRTI and wheezing in 12-14-month-old children in Spain (Gascon et al., 2012). In contrast, prenatal DDE concentrations were not associated with LRTIs in 18-month-old Mexican boys (Cupul-Uicab et al., 2014).

Few studies have examined the associations between pesticides other than DDE/DDT and respiratory outcomes in children. In an analysis of National Health and Nutrition Examination Survey (NHANES) 1999–2008, organophosphate (OP) metabolite concentrations in school-age children were not associated with current wheeze (Perla et al., 2015). However, a cross-sectional analysis of school-age children with asthma living in an agricultural community in Washington State found that short-term exposure to OPs was associated with a higher risk of asthma morbidity (Benka-Coker et al., 2019). In addition, a birth cohort study of California children living near agricultural fields found these OP metabolite concentrations in the second half of pregnancy and in childhood to be associated with respiratory symptoms at ages 5 and 7 years (Raanan et al., 2015) and/or decreased lung function at 7 years (Raanan et al., 2016). Prenatal exposure to piperonyl butoxide, a synergist for residential pyrethroid insecticides, was associated with cough at age 5–6 years in a New York City birth cohort (Liu et al., 2012). In a cross-sectional study in France, higher urinary ETU concentrations, a marker of exposure to bisdithiocarbamate fungicides, were associated with asthma and rhinitis symptoms in children aged 3–10 years (Raherison et al., 2019). To our knowledge, no published study has examined the association of prenatal exposure to current-use pesticides with respiratory symptoms and infections during infancy.

In Costa Rica, residents living in banana growing regions are exposed to a variety of pesticides including fungicides (e.g., mancozeb), insecticides (e.g., OP chlorpyrifos, cypermethrin), and herbicides (e.g., 2,4-D). These exposures result in measurable concentrations of pesticides and their metabolites in the residents, including children (van Wendel de Joode et al., 2012) and pregnant women (van Wendel de Joode et al., 2014). Using biological monitoring data from the Infants' Environmental Health ('Infantes y Salud Ambiental', ISA) study, a prospective birth cohort study of pregnant women and their children living near banana plantations, we evaluated the impact of prenatal pesticide exposure on respiratory health in the first year of life.

2. Methods

2.1. Study population

Pregnant women were enrolled in the ISA study from March 2010 and June 2011 (Mora et al., 2014; van Wendel de Joode et al., 2014). Of 451 women enrolled in the study, 22 (5%) had a miscarriage or stillbirth and 69 (15%) were lost to follow-up before the one-year study visit. Of the remaining 360 mother-child pairs, 355 (99%) singleton liveborn infants had maternal urinary pesticide concentrations measured during pregnancy and available information on respiratory outcomes in the first year of life. Mother-child pairs included in these analyses (n = 355) did not differ significantly from the initial cohort (n = 451) (van Wendel de Joode et al., 2014) on their attributes, including maternal education, parity, household income, and prenatal specific gravity-corrected urinary pesticide concentrations.

The Ethical and Scientific Committee of the Universidad Nacional in Costa Rica approved all study protocols. All mothers provided written informed consent at enrollment and additional informed consent was obtained from the parents or legal guardians of participants aged < 18 years.

2.2. Data collection

We interviewed women during pregnancy (one to three times depending on their gestational age at enrollment; median at the first, second, and third visit = 19, 30, and 33 weeks of gestation, respectively), shortly after delivery (median = 7 weeks' postpartum), and when their children were 11–19 months old (median = 13.2 months; one-year study visit). We collected socio-demographic data, such as maternal age, education, parity, and household income, at the baseline interview. We also gathered information on maternal occupational status, smoking habits, medical conditions, medications, and obstetric ultrasounds at each interview. We abstracted data completed by hospital/clinic personnel from prenatal (e.g., ultrasounds) and delivery (e.g., length of gestation) medical records provided to the study participants. We estimated gestational age at birth using the last menstrual period date, information from early ultrasounds (< 14 weeks of gestation), and medical record estimates (Mora et al., 2015).

2.3. Respiratory outcomes

We evaluated two respiratory outcomes: physician- or nurse-confirmed diagnosis of LRTIs and wheeze during the first year of life. Information about these outcomes was obtained from mothers through questionnaires when children were 11–19 months old. Occurrence of a LRTI episode was defined as a positive answer to one of the following two questions: "Has a doctor or nurse ever told you that your child has pneumonia?" or "Has a doctor or nurse ever told you that your child has bronchiolitis or bronchitis?". Children with negative answers to both questions were defined as not having LRTI. Wheezing during the first year of life was defined as a positive answer to the question "Since he/she were born, has your child ever experienced whistling or wheezing from the chest?". Questions were extracted from Spanish version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (ISAAC, 1998) and have been previously used in other cohort studies (Gascon et al., 2012). LRTI questions were administered to mothers of all infants who were assessed at the one-year study visit (n = 355), whereas the wheezing question was administered to only 272 mothers (because it was added to the questionnaire after the study visits had already started).

2.4. Urinary pesticide metabolites measurements

We collected maternal urine samples one to three times during pregnancy (at the same time as pregnancy interviews) in 100 mL

beakers (Vacuette®, sterile), aliquoted them into 15 mL tubes (PerformR™ Centrifuge tubes, Labcon®, sterile), and then stored them at −20 °C until shipment to the Division of Occupational and Environmental Medicine at Lund University, Sweden, for analysis. A total of 93 women provided three samples during pregnancy, 222 women provided two samples, and 40 provided only one. Samples were analyzed for metabolites of fungicides [ethylenethiourea (ETU, metabolite of mancozeb), hydroxypyrimethanil (OH-PYR, metabolite of pyrimethanil), and 5-hydroxythiabendazole (5-OH-TBZ, metabolite of thiabendazole)] and OP insecticides [3,5,6-trichloro-2-pyridinol (TCPy, metabolite of chlorpyrifos) used in banana plantations (Table S1). Urine specimens were also analyzed for metabolites of common synthetic pyrethroids used in vector control programs and at home, but not in banana: 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCCA, metabolite of permethrin, cypermethrin, and cyfluthrin); and 3-phenoxybenzoic acid (3PBA, metabolite of permethrin, cypermethrin, cyfluthrin, deltamethrin, allethrin, resmethrin, and fenvalerate). The herbicide 2,4-D, used to control broadleaf in pasture and rice, was also measured in urine samples.

Urinary metabolites were measured using a liquid chromatography mass spectrometer (LC-MS/MS; UFLCRX; Shimadzu Corporation) with a triple quadrupole linear ion trap (QTRAP 5500; AB Sciex) (Ekman et al. 2013, 2014; Faniband et al., 2019). For ETU analyses, duplicate urine samples were added with internal standards, hydrolyzed using basic buffer, and then analyzed using two-dimensional LC-MS/MS methodology (Ekman et al., 2013). For all other analyses, duplicate urine specimens were added with internal standards and hydrolyzed using sulfatase/glucuronidase enzyme thereafter the metabolites were extracted from the urinary matrix using solid phase extraction (Norén et al., 2020). Average concentrations of the duplicate samples were used in further calculations. Between-run and between-batch precisions were 4–18% and 8–19%, respectively.

Pesticide metabolite concentrations were normalized for dilution using the formula $M_{SG} = M \times [(1.017 - 1) - (SG - 1)]$, where M_{SG} is the specific gravity-corrected metabolite concentration (μg/L), M is the observed metabolite concentration (μg/L), SG is the specific gravity of the urine sample, and 1.017 kg/L is the average specific gravity for all urine samples included in these analyses (n = 763). Urinary specific gravity (kg/L) was determined using a hand refractometer.

2.5. Statistical analyses

We calculated descriptive statistics and distributional plots for all variables. We then estimated bivariate associations between biomarkers of exposure, outcomes, and covariates using t-tests and chi-square tests. We also estimated correlations between specific gravity-corrected urinary pesticide metabolite concentrations using Spearman's correlation coefficients (r_s).

We examined associations of maternal urinary pesticide metabolite concentrations with respiratory outcomes using multivariable logistic regression models. We examined three windows of exposure: (i) first half of pregnancy (≤20 weeks of gestation, n = 194), (ii) second half of pregnancy (> 20 weeks of gestation, n = 343), and (iii) average over the course of pregnancy (n = 355). Our primary analyses focused on evaluating those in the top quartile [≥75th percentile (P75)] vs. all other concentrations (< P75), but we also ran our models with our exposures modeled as continuous variables (i.e., $\log_{10}$-transformed specific gravity-corrected urinary pesticide metabolite concentrations). We selected our covariates *a priori* using directed acyclic graphs based on previous literature (Cupul-Uicab et al., 2014; Gascon et al. 2012, 2014): maternal age, maternal education, parity, gestational age at birth, and infant sex. We imputed missing values for covariates (all < 5% missing) using data from the nearest available study visit. If values for a missing covariate were not available from an earlier or later study visit, we randomly selected a value from the dataset (n = 2 participants missing parity; n = 2 participants missing maternal smoking during pregnancy for sensitivity analyses).

We conducted sensitivity analyses to assess the robustness of our results and better understand our exposure-outcome associations. Because maternal smoking during pregnancy has been identified as a risk factor for infant wheezing and LRTI (McEvoy and Spindel, 2017) but the low prevalence of maternal smoking during pregnancy in our study population (n = 15) prevented us from conducting stratified analyses and adjusting for this variable, we excluded these mothers from our models. In addition, we ran our main models only with mothers who had exposure data for both the first and second half of pregnancy (n = 182 for LRTI and 161 for wheeze). We also clustered mother-infant pairs into four groups, based on urinary ETU concentrations during the first and second half of pregnancy dichotomized at the P75 of the two distributions: a) low ETU during first half/low ETU during second half, representing concordant low exposures; b) high ETU during first half/low ETU during second half; c) low ETU during first half/high ETU during second half; and d) high ETU during first half/high ETU during second half, representing concordant high exposures. Using multivariable regression models, we estimated the associations of this categorical variable with LRTI and wheezing during the first year of life. All statistical analyses were performed using Stata (version 14.2; StataCorp LLC) and R (version 3.1.2; R Development Core Team).

3. Results

Ten percent of the children had at least one LRTI and 39% had at least one episode of wheezing during their first year of life (Table 1). About 71% of children with history of a LRTI also had wheezing. LTRI and wheezing were both associated with maternal smoking both during pregnancy and during the first year of life. Mothers included in our analyses were relatively young at the time of enrollment (median age = 22.3 years; range = 15–44), predominantly Costa Rican-born (83%) and multiparous (64%), had no history of asthma (87%), and lived below the Costa Rican poverty line at the time of enrollment (59%). Only 4% of mothers reported smoking during pregnancy. Pesticides were detected in the urine of all women, with ETU, TCPy, 3PBA, and 2,4-D detected in all samples (Table 2). Median (P25-P75) specific gravity-corrected urinary ETU, TCPy, and 3PBA concentrations averaged during pregnancy were 3.3 (2.4–4.9) ng/mL, 1.8 (1.3–2.5) ng/mL, and 0.8 (0.5–1.3) ng/mL, respectively. Urinary pesticide metabolites measured during the same window of exposure were weakly to moderately correlated (r_s for first half of pregnancy = 0.03–0.34; r_s for second half of pregnancy = 0.01–0.20; r_s for pregnancy average = 0–0.24), except for pyrethroid metabolites 3PBA and DCCA, which were highly correlated during all exposure periods (r_s = 0.79–0.84; Table S2).

We observed that most associations of the seven prenatal urinary pesticide metabolites [categorized in high (≥P75) and low (< P75)] with respiratory outcomes during the first year of life hovered around the null (Table 3). However, we found that high urinary ETU concentrations during the first half of pregnancy were associated with increased odds of LRTI (OR = 2.45; 95% CI: 0.96, 6.26). We also observed that high urinary ETU concentrations during the second half of pregnancy were associated with decreased odds of wheezing (OR = 0.50; 95% CI: 0.26, 0.96). We observed similar associations when we ran our models with our exposures modeled as $\log_{10}$-transformed specific gravity-corrected urinary pesticide metabolite concentrations (OR for ETU concentrations during the first half of pregnancy and LRTI = 8.20; 95% CI: 1.66, 40.59; OR for ETU concentrations during the second half of pregnancy and wheezing = 0.37; 95% CI: 0.13, 1.02; Table S3). Our effects estimates did not change appreciably when we excluded mothers who reported smoking during pregnancy (Table S4).

Table 1
Characteristics of study population by respiratory outcomes at one year of age, ISA study, Matina County, 2010–2013 [n (%) or median (P25-P75)].

	LRTI (n = 355)		Wheeze (n = 272)[a]	
	Never	Ever	Never	Ever
All children	318 (89.6)	37 (10.4)	166 (61.0)	106 (39.0)
Child characteristics				
Child's sex				
Boy	154 (48.4)	24 (64.9)	85 (51.2)	54 (50.9)
Girl	164 (51.6)	13 (35.1)	81 (48.8)	52 (49.1)
Low birth weight (< 2,500 g)				
No	305 (95.9)	35 (94.6)	161 (97.0)	100 (94.4)
Yes	8 (2.5)	2 (5.4)	4 (2.4)	3 (2.8)
Missing	5 (1.6)	0 (0.0)	1 (0.6)	3 (2.8)
Preterm birth (< 37 weeks)				
No	298 (93.7)	34 (91.9)	154 (92.8)	103 (97.2)
Yes	20 (6.3)	3 (8.1)	12 (7.2)	3 (2.8)
Breastfeeding duration				
≤6 months	71 (22.3)	11 (29.7)	44 (26.5)	24 (22.6)
> 6 months	247 (77.7)	26 (70.3)	122 (73.5)	82 (77.4)
Age at outcome assessment (months)	13.1 (12.4–14.7)	13.0 (12.2–14.7)	13.3 (12.5–15.3)	13.8 (12.6–15.1)
Maternal characteristics				
Age at enrollment (years)	22.1 (19.1–28.2)	23.5 (20.9–28.6)	21.4 (18.5–25.8)	23.0 (19.7–29.1)
Country of birth				
Costa Rica	266 (83.7)	30 (81.1)	134 (80.7)	90 (84.9)
Other	52 (16.3)	7 (18.9)	32 (19.3)	16 (15.1)
Education				
≤6th grade	169 (53.1)	12 (32.4)	82 (49.4)	57 (53.8)
> 6th grade	149 (46.9)	25 (67.6)	84 (50.6)	49 (46.2)
Parity				
0	117 (36.8)	9 (24.3)	66 (39.8)	34 (32.1)
≥1	199 (62.6)	28 (75.7)	99 (59.6)	72 (67.9)
Missing	2 (0.6)	0 (0.0)	1 (0.6)	0 (0.0)
Agricultural work at enrollment				
No	289 (90.9)	35 (94.6)	155 (93.4)	93 (87.7)
Yes	29 (9.1)	2 (5.4)	11 (6.6)	13 (12.3)
Agricultural work at one-year visit				
No	241 (75.8)	28 (75.7)	130 (78.3)	75 (70.8)
Yes	77 (24.2)	9 (24.3)	36 (21.7)	31 (29.2)
History of asthma				
No	274 (86.2)	34 (91.9)	144 (86.8)	91 (85.9)
Yes	44 (13.8)	3 (8.1)	22 (13.2)	15 (14.1)
Smoking during pregnancy				
No	306 (96.2)	33 (89.2)	163 (98.2)	95 (89.6)
Yes	11 (3.5)	4 (10.8)	3 (1.8)	10 (9.4)
Missing	9 (0.3)	0 (0.0)	0 (0.0)	1 (1.0)
Cotinine levels during pregnancy (LOD = 1 ng/mL)				
< LOD	259 (81.4)	30 (81.1)	137 (82.5)	83 (78.3)
≥LOD	48 (15.1)	7 (18.9)	25 (15.0)	17 (16.0)
Missing	11 (3.5)	0 (0.0)	4 (2.4)	6 (5.7)
Smoking during the year after delivery				
No	309 (97.2)	34 (91.9)	164 (98.8)	98 (92.5)
Yes	9 (2.8)	3 (8.1)	2 (1.2)	8 (7.5)
Household characteristics				
Income at enrollment[b]				
> Poverty	125 (39.3)	17 (46.0)	73 (44.0)	41 (38.7)
< Poverty and > extreme poverty	129 (40.6)	12 (32.4)	62 (37.3)	43 (40.6)
< Extreme poverty	60 (18.9)	8 (21.6)	29 (17.5)	21 (19.8)
Missing	4 (1.2)	0 (0.0)	2 (1.2)	1 (0.9)

Abbreviations: LOD, limit of detection; LRTI, lower respiratory tract infection; *n*, number of children; P25, 25th percentile; P75, 75th percentile.
[a] Wheezing question was administered to only 272 mother-child pairs because it was added to the questionnaire after the one-year visits had already started.
[b] Costa Rican poverty and extreme poverty lines in 2011: US$141 and US$69 per capita per month.

When we ran our main models only with mothers who had exposure data for both the first and second half of pregnancy, we found that high urinary ETU concentrations during the first half of pregnancy remained associated with increased odds of LRTI (OR = 2.69; 95% CI: 1.03, 7.05; n = 182; data not shown in tables or figures). We also observed that high urinary ETU concentrations during the second half of pregnancy were no longer associated with decreased odds of wheezing among these women (OR = 0.51; 95% CI: 0.22, 1.21; n = 161). Similarly, when we clustered mother-infant pairs into four groups based on their urinary ETU concentrations during the first and second half of pregnancy, we observed that pairs with high ETU during first half/low ETU during second half had increased odds of LRTI during the first year of

Table 2
Distribution of prenatal pesticide metabolites (specific gravity-adjusted, ng/mL) concentrations in the study population, ISA study, 2010–2013.

Pesticide metabolite	% > LOD	Min	Percentile			Max
			25th	50th	75th	
1st half of pregnancy (n = 194)						
ETU	100	0.58	2.14	3.40	4.97	31.06
TCPy	100	0.28	1.06	1.62	2.46	50.01
3PBA	100	0.07	0.42	0.72	1.26	32.61
2,4-D	100	0.04	0.17	0.27	0.48	3.50
DCCA	100	0.06	0.60	1.16	2.02	45.77
OH-PYR	85	< LOD	0.15	0.32	0.86	946.36
5-OH-TBZ	63	< LOD	0.02	0.06	0.29	144.73
2nd half of pregnancy (n = 343)						
ETU	100	0.65	2.21	3.25	4.70	127.38
TCPy	100	0.41	1.21	1.77	2.59	91.10
3PBA	100	0.06	0.44	0.73	1.34	16.81
2,4-D	100	0.05	0.22	0.31	0.55	159.21
DCCA	99	0.13	0.68	1.20	2.21	18.06
OH-PYR	92	< LOD	0.19	0.49	1.25	368.55
5-OH-TBZ	72	< LOD	0.02	0.09	0.47	644.46
Pregnancy average (n = 355)						
ETU	100	0.81	2.38	3.35	4.90	127.38
TCPy	100	0.41	1.31	1.75	2.54	62.96
3PBA	100	0.10	0.49	0.79	1.31	16.96
2,4-D	100	0.09	0.23	0.33	0.53	79.76
DCCA	100	0.15	0.75	1.30	2.30	23.56
OH-PYR	94	< LOD	0.21	0.56	1.33	368.55
5-OH-TBZ	76	< LOD	0.03	0.11	0.58	339.00

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; DCCA, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid; ETU, ethylenethiourea; ICC, intraclass correlation coefficient; LOD, limit of detection; n, number of children; OH-P, 3-hydroxypyrimetanil; 5-OH-TBZ, 5-hydroxythiabendazol; 3-PBA, 3-phenoxybenzoic acid; SD, standard deviation; TCPy, 3,5,6-trichloro-2-pyridinol.

Table 3
Adjusted associations [OR (95% CI)] for prenatal pesticide metabolites (high vs. low)[a] and respiratory outcomes at one year of age, ISA study, 2010–2013.

Pesticide metabolites	LRTI	Wheezing
First half of pregnancy	n = 194	n = 172
ETU	2.45 (0.96, 6.26)	1.01 (0.48, 2.12)
TCPy	1.36 (0.50, 3.68)	0.73 (0.34, 1.56)
3PBA	1.47 (0.54, 4.01)	1.52 (0.73, 3.16)
2,4-D	1.21 (0.43, 3.40)	0.79 (0.37, 1.68)
DCCA	1.90 (0.72, 5.01)	1.71 (0.82, 3.57)
OH-PYR	1.60 (0.58, 4.38)	1.02 (0.48, 2.16)
5-OH-TBZ	0.34 (0.09, 1.26)	1.50 (0.74, 3.03)
Second half of pregnancy	n = 343	n = 261
ETU	0.87 (0.37, 2.05)	0.50 (0.26, 0.96)
TCPy	0.60 (0.24, 1.53)	0.82 (0.45, 1.50)
3PBA	1.40 (0.64, 3.05)	1.23 (0.68, 2.22)
2,4-D	1.45 (0.67, 3.11)	0.87 (0.47, 1.60)
DCCA	0.92 (0.40, 2.10)	0.76 (0.40, 1.47)
OH-PYR	0.93 (0.41, 2.13)	0.71 (0.37, 1.33)
5-OH-TBZ	1.03 (0.46, 2.30)	0.69 (0.38, 1.28)
Pregnancy average	n = 355	n = 272
ETU	1.50 (0.70, 3.19)	0.69 (0.37, 1.28)
TCPy	0.84 (0.36, 1.95)	0.86 (0.48, 1.54)
3PBA	1.64 (0.78, 3.47)	1.07 (0.60, 1.90)
2,4-D	1.48 (0.69, 3.14)	0.87 (0.48, 1.59)
DCCA	1.07 (0.49, 2.36)	0.74 (0.39, 1.38)
OH-PYR	1.49 (0.70, 3.18)	0.83 (0.45, 1.53)
5-OH-TBZ	0.98 (0.45, 2.16)	0.80 (0.45, 1.45)

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; DCCA, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid; ETU, ethylenethiourea; LRTI, lower respiratory tract infection; n, number of children; OH-P, 3-hydroxypyrimetanil; 5-OH-TBZ, 5-hydroxythiabendazol; OR, odds ratio; 3-PBA, 3-phenoxybenzoic acid; TCPy, 3,5,6-trichloro-2-pyridinol.
Models adjusted for maternal age, education, parity, gestational age at birth, and child sex.
[a] Pesticide exposure categorized as high (≥75th percentile) and low (≥75th percentile, reference category).

life, compared to mother-child pairs with low ETU during first half/low ETU during second half of pregnancy (OR = 3.08; 95% CI: 0.98, 9.67; Fig. 1). Odds of wheezing were not associated with urinary ETU concentrations in our four-group analyses (e.g., OR for mother-infant pairs with low ETU during first half/high ETU during second half compared to the low concordant exposure group = 0.62; 95% CI: 0.22, 1.70; Fig. 1).

4. Discussion

Our findings suggest that prenatal exposure to mancozeb, as indicated by urinary ETU concentrations in women during pregnancy, is associated with respiratory outcomes in the first year of life. We observed that infants in the highest quartile of maternal urinary ETU concentrations during the first half of pregnancy had increased odds of LRTI, compared to those in the lowest three quartiles. We also found that infants in the highest quartile of maternal urinary ETU concentrations during the second half of pregnancy had decreased odds of wheezing, compared to those in the lowest three quartiles, but this association was attenuated when we included in our analyses only mothers with complete exposure information and when we accounted for maternal ETU concentrations during the first half of pregnancy. It could be possible that exposure to current-use pesticides during the first half of pregnancy is more important than exposure during the second half; however, further research is warranted to assess the possibility of susceptible period(s) during pregnancy and the mechanisms by which exposure to current-use pesticides may affect respiratory system development.

Our findings on ETU and respiratory health are somewhat consistent to the only published study to date on the association of bisdithiocarbamates fungicides exposure and respiratory outcomes in children (Raherison et al., 2019). This cross-sectional study conducted in France found that higher urinary ETU concentrations were associated with asthma and rhinitis symptoms in children aged 3–10 years. This was a relatively small study (n = 66) that examined health effects in older children (mean age = 7.5 years). The creatinine-corrected urinary ETU concentrations in their study population (medians in Phases I and II = 0.4 and 0.7 μg/g creatinine, respectively; n = 96) were lower than those observed in the present study (median throughout pregnancy = 3.1 μg/g creatinine), but were higher than those observed in the US general population sample of children aged 6–11 years from the NHANES 2007–2008 study (median < LOD, P95 = 1.0 μg/g creatinine, n = 382) (Centers for Disease Control and Prevention (CDC) 2019). The potential mechanism of bisdithiocarbamates exposure on respiratory health is not well understood; nevertheless, evidence from occupational studies suggests that these fungicides could affect respiratory function by modulating the immune system, inducing macrophage activation, and enhancing the inflammatory response (Colosio et al., 1996; Corsini et al., 2005; Weis et al., 2019). Given the widespread use of mancozeb worldwide, more research studies are needed to understand better the specific mechanisms by which bisdithiocarbamates interfere with the immune and respiratory systems.

Although no published study has assessed the association of exposure to current-use pesticides other than bisdithiocarbamate

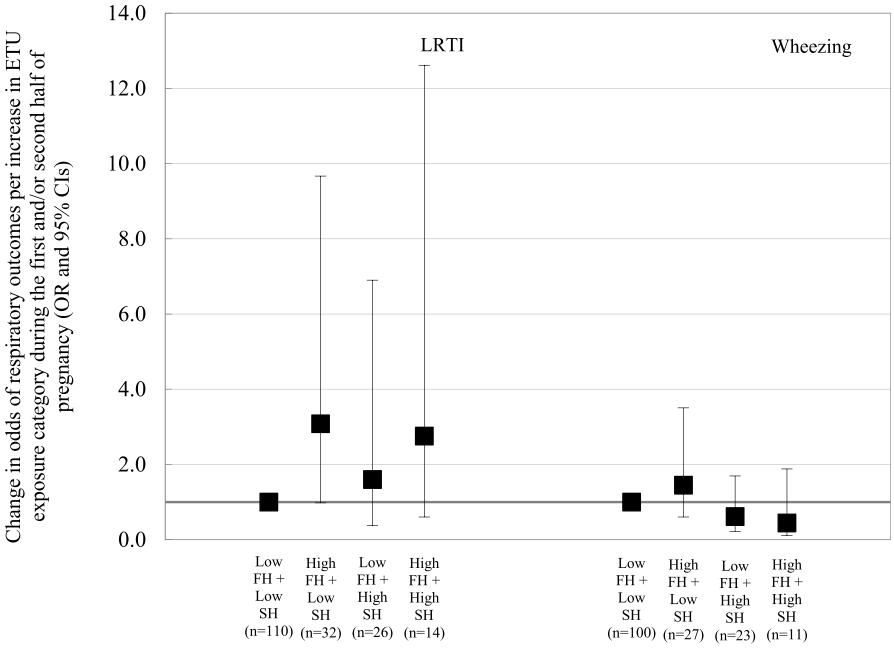

Fig. 1. Adjusted associations of urinary ETU concentrations during the first (FH) and second (SH) half of pregnancy with respiratory outcomes at one year of age, ISA study, 2010–2013. Models adjusted for maternal age, education, parity, gestational age at birth, and child sex. High indicates above or at the 75th percentile (P75); low indicates below P75. P75 for ETU concentrations during the first half of pregnancy = 3.40 ng/mL; P75 for ETU concentrations during the second half of pregnancy = 3.25 ng/mL. Low FH + Low SH: reference category. *Abbreviations*: ETU, ethylenethiourea; LRTI, low respiratory tract infections.

fungicides with respiratory health in the first year of life, a few studies have examined the association of OP exposure with respiratory outcomes in school-age children (Benka-Coker et al., 2019; Perla et al., 2015; Raanan et al. 2015, 2016). These studies have reported inconsistent results, possibly due to differences in their study design (e.g., cross-sectional vs. prospective cohort), sources of pesticide exposure (e.g., diet vs. drift from agricultural fields), windows of exposure (e.g., prenatal vs. childhood), and/or exposure assessment methods (e.g., non-specific vs. OP-specific metabolites). For example, in a cross-sectional study of U.S. children aged 5–16 years, OP exposure, as indicated by non-specific dialkyl phosphate (DAP) metabolites in urine, was not associated with parent-reported asthma (n = 2,777) (Perla et al., 2015). In contrast, a birth cohort study of children living near agricultural fields in California observed that higher urinary DAP metabolites in the second half of pregnancy and early childhood (0.5–5 years) were associated with parent-reported respiratory symptoms at ages 5 and 7 years (n = 359) (Raanan et al., 2015) and/or decreased lung function at 7 years (n = 279) (Raanan et al., 2016). Additionally, a very small cross-sectional study of children with asthma aged 6–16 years and living in an agricultural community in Washington State (n = 16), found that higher urinary DAPs metabolites were associated with increased urinary leukotriene E4, a marker of asthma exacerbation (Benka-Coker et al., 2019). In our study, we did not measure urinary DAP metabolites. We measured urinary TCPy, a metabolite specific to OP insecticide chlorpyrifos, in maternal samples collected during pregnancy, but did not find an association with respiratory outcomes in the first year of life; nor did we observe associations of prenatal exposure to pyrimethanil, thiabendazole, common synthetic pyrethroids, and 2,4-D with LRTI and wheezing during the first year of life. In the present study, we did not assess exposure to elemental sulfur or piperonyl butoxide both pesticides and/or pesticide ingredients that have been previously associated with respiratory outcomes in children (Liu et al., 2012; Raanan et al., 2017).

Our study has limitations, mostly related to the assessment of respiratory outcomes. We did not obtain medical records to corroborate the physician- or nurse-confirmed diagnosis of LRTI or wheezing episodes that mothers reported via questionnaire; thus, non-differential outcome misclassification may have occurred. In addition, we examined respiratory outcomes during the first year of life, which may be too early to identify long-lasting respiratory effects of prenatal exposures to environmental toxicants. At the present time, further respiratory assessments of the ISA study participants are being conducted to determine if the exposure-outcome associations observed during the first year of life persist throughout childhood. In our study, we cannot rule out the possibility that selection bias could have arisen from loss to follow-up. Lastly, we recognize that we conducted multiple comparisons, which could have led to statistically significant associations by chance, but we tried to look for patterns in our results rather than to highlight isolated findings.

The present study has considerable strengths, perhaps most notable among them being its longitudinal nature. We measured urinary pesticide metabolites concentrations in maternal samples collected repeatedly during pregnancy – which is a strength considering that the metabolites that we measured reflect short-term pesticide exposures (Lindh et al., 2008; Nolan et al., 1984) – and evaluated respiratory outcomes at age 1. As in any epidemiologic study, the exposure-outcome associations that we found in our study could be attributable to uncontrolled confounders, but we were able to assess or adjust for several important factors, including maternal characteristics.

To our knowledge, ours is the first study to examine the association of prenatal exposure to current-use pesticides with respiratory symptoms and infections during the first year of life. Early life respiratory outcomes can have long-term consequences for respiratory health (Busse et al., 2010; Jackson et al. 2008, 2016; Jackson, 2014; Lemanske et al., 2005; Liu et al., 2017; Lodge et al., 2014; Rubner et al., 2017). For example, viral wheezing respiratory illnesses in infancy and early childhood life have been associated with an increased risk of asthma at school age (Jackson et al., 2008) and adolescence (Rubner et al., 2017). Early life viral wheezing illnesses have also been associated with decreased lung function at age 8 years (Guilbert et al., 2011). Previous studies have shown that the respiratory and allergic disease risk profiles in Costa Rica are similar to those reported in industrialized countries with a Western lifestyle (Celedon et al., 2002) and that children in Costa Rica have one of the highest prevalence of wheeze worldwide (Lai et al., 2009). In our study population, 39% of children had at least one episode of wheezing during their first year of life, whereas 27% of children from eight European cohorts (Gascon et al., 2014) and 26% of children from a New York City birth cohort (Donohue et al., 2008)

experienced wheezing by ages 1.5 and 3 years, respectively. In contrast, only 10% of the children included in our study had developed at least one LRTI episode during their first year of life, whereas 35% of the children from a large Spanish birth cohort (Gascon et al., 2012) and 19% of the children from a Mexican birth cohort (Cupul-Uicab et al., 2014) experienced at least one LRTI by ages 12–14 and 18 months, respectively. Further studies should examine how prevalence differences in respiratory outcomes during infancy and early-life exposure to pesticides can influence long-term respiratory health effects.

5. Conclusions

Prenatal exposure to mancozeb, but not to other current-use pesticides, was associated with respiratory outcomes during the first year of life in infants living near banana plantations in Costa Rica. Our results are biologically plausible, given the immunomodulatory effects of bisdithiocarbamate fungicides observed in occupational studies, and are, to some degree, consistent with the only published study to date on the association of bisdithiocarbamates fungicides exposure and respiratory outcomes in children. Our findings provide additional evidence supporting an association between prenatal pesticide exposure and respiratory health in children and are important due to the widespread use of pesticides in agriculture (Food and Agriculture Organization of the United Nations (FAO) 2019) and increasing prevalence of chronic respiratory diseases in children worldwide (Pearce et al., 2007; Zar and Ferkol, 2014).

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CRediT author statement

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Declaration of competing interest

None of the other authors declares any actual or potential competing financial interests.

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Appendix A. Supplementary data

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Risk assessment concepts and approaches for indoor air chemicals in Japan

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ABSTRACT

Individuals living in general indoor environments are exposed to a greater variety of chemical pollutants, albeit at lower concentrations, compared with industrial workers in occupational environments. These pollutants can result in a variety of adverse health effects, including those affecting the respiratory, neurological, reproductive, dermatologic, and cardiovascular systems. In Japan, indoor air quality guidelines have been established for 13 chemicals since 1997, and these developments have continued on the basis of scientific discussions in the Committee on Indoor Air Pollution (CIAP) that was set up by the Ministry of Health, Labour and Welfare. However, the types and concentrations of these pollutants have been observed to be inconsistent over time due to lifestyle changes and the development of novel household products and building materials. Therefore, continuing the monitoring of indoor chemicals and the development of indoor air quality guidelines for substances that pose potential high health risks are essential for the protection of public health. In indoor environments, there are multiple media by which humans come in contact with indoor chemicals and multiple exposure pathways that can affect human health, particularly for semi-volatile organic compounds (SVOCs). This is defined as aggregate exposure. Furthermore, combined exposure to multiple low-level pollutants occurs in indoor environments. In this article, a comprehensive overview of the indoor air quality guidelines in Japan and assessment approaches for developing indoor air quality guidelines is provided. In addition, future issues facing approaches for indoor chemicals, including aggregate exposure to SVOCs and combined exposure to multiple pollutants with common toxicological effects in indoor environments, are discussed.

1. Introduction

Indoor air quality (IAQ) is an important determinant of human health. People in modern societies spend more than 90% of their time indoors, i.e., in their homes, workplaces, schools, transportation, and public spaces (Brasche and Bischof, 2005; Leech et al., 2002). Individuals living in indoor environments are typically exposed to a greater variety of chemical pollutants, albeit at lower concentrations, compared with industrial workers in occupational environments. These pollutants can have a variety of adverse health effects, including those affecting the respiratory, neurological, reproductive, dermatologic, and cardiovascular systems (Wu et al., 2007). Hence, a high level of protection against adverse health effects resulting from inadequate IAQ should be assured.

In the 1990s, public health problems caused by chemical indoor air pollutants were a cause of considerable public concern in Japan. After conducting extensive exposure assessments, the Ministry of Health, Labour and Welfare (MHLW) established IAQ guidelines for 13 chemicals, including formaldehyde, toluene, and xylene from 1997 to 2002, based on scientific discussions in the Committee on Indoor Air Pollution (CIAP) (Azuma et al., 2007, 2008; MHLW, 2000a, 2000b; MHLW, 2001; MHLW, 2002). In addition, the National Building Codes and formaldehyde emission standards used to monitor building materials were revised (Azuma et al., 2008). However, neither the types nor the concentrations of chemicals found indoors are consistent. Changes occur day-to-day, month-to-month, year-to-year, and decade-to-decade with changes in lifestyle, the development of novel household products and building materials, and the development of new measurement technologies (Weschler, 2009). Adverse health effects caused by semi-volatile organic compounds (SVOCs) have been reported over the past

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decade (Bornehag and Nanberg, 2010; Jaakkola and Knight, 2008; Lyche et al., 2009). Therefore, the MHLW started to establish new (or update) IAQ guidelines from 2012, and the CIAP was relaunched. Extensive exposure assessments for housing were performed in Japan, and the CIAP proposed a new approach to develop IAQ guidelines based on these health risk assessments.

This article aims to provide a comprehensive overview of the Japanese approaches to regulate the levels of indoor air chemicals. In particular, we highlight the risk assessment concepts used to reduce adverse health effects of indoor air chemicals. In addition, future issues regarding multi-route and multi-media exposure to SVOCs as well as multiple exposures to indoor air chemicals and their combined health effects are discussed.

2. Approaches to ensuring adequate IAQ

2.1. Basic concepts used to regulate indoor air chemicals

There are several important barriers to developing policies to improve the air quality in indoor environments. One major difficulty in regulating indoor air is that it is not the responsibility of a single department or ministry and, in many countries, no specific laws comprehensively addressing the subject exist. Regulation can also affect the privacy of individuals (Seifert, 1992). In addition, a single measurement of indoor chemical pollutants does not represent the air quality in a particular indoor environment because indoor chemical pollutant concentrations change in accordance with indoor temperature and time-dependent reductions in source emissions. The concentration of an indoor chemical pollutant depends on the relationships between the volume of air contained in the indoor space, the rate of production or release of the pollutant, the rate of removal of the pollutant from the air via reaction or settling, the rate of air exchange with the outside atmosphere, and the outdoor pollutant concentration (Jones, 1999). Furthermore, indoor air chemicals emanate from a range of sources. They are emitted by the fabric of buildings and can also be a by-product of the activities that are undertaken within them.

Sources can be broadly classified as being associated with the activities of building occupants and other biological sources, the combustion of substances for heating or fuel, and emissions from building materials. For some contaminants, infiltration from outside, either through water, air, or soil, can also be a significant source (Jones, 1999; Wu et al., 2007). Therefore, the indoor environment does not lend itself to the regulatory approach typically used to limit ambient air pollutants, i.e., the setting of standards. Thus, guideline values, rather than standards, are used to regulate IAQ (Harrison, 2002; Levin, 1998; Seifert, 1992; Seifert et al., 1999).

Guideline values for specific indoor air chemicals have been developed by the World Health Organization (WHO) Regional Office for Europe (WHO Europe, 2010) and agencies or ministries of health or environment in several countries such as Germany (Fromme et al., 2019), Canada (GC, 2018), France (ANSES, 2018), and Japan. This strategy both protects the public from health effects due to indoor chemical pollutants and promotes the prevention of pollution. Furthermore, such guideline values, together with appropriate modeling, can serve to limit emissions, especially those from building materials and household products (Seifert, 1992).

2.2. IAQ guidelines in Japan

2.2.1. Concepts of IAQ guidelines

In order to protect public health, unnecessary exposure to indoor chemicals should be minimized and chemicals should be safely and appropriately used so that they have no adverse effects on human health (MHLW, 2019). The guideline values for indoor air concentrations are set so that, according to currently available scientific knowledge, no adverse health effects would be expected to occur in humans, even if exposures to the chemicals at the levels decided continue throughout life. IAQ guidelines are applied to all indoor spaces, including housing, offices, medical facilities, welfare facilities, schools, public facilities, and transportation facilities, but exclude specific spaces such as industrial plants. These values may be revised in the future as necessary depending on further available knowledge and/or progress in international assessment based on such scientific knowledge (MHLW, 2000a, 2000b; MHLW, 2001; MHLW, 2002; MHLW, 2019).

2.2.2. Toxicologically based guideline values for 13 chemicals

In 2000, as a means to set the priority of indoor air chemicals for which guideline values should be established, the CIAP considered the following six criteria (MHLW, 2000a):

(1) Guideline values for indoor air chemicals that have already been set by other governmental agencies or international organizations; for example, the WHO Air Quality Guidelines.
(2) Air pollutants for which indoor concentrations are higher than those outdoors because of indoor emission sources in residential environments.
(3) Public complaints about indoor air chemicals such as total volatile organic compounds (TVOC).
(4) New regulations for indoor air chemicals already established by other foreign governments; for example, chlorpyrifos or diazinon by the United States Environmental Protection Agency.
(5) Rules covering major uses of chemicals in construction, such as solvents, adhesives, insecticides, plasticizers, and termiticides.
(6) Rules covering major chemical structural categories of volatile organic compounds (VOCs), including aldehydes, ketones, aromatic hydrocarbons, halocarbons, alkanes, terpenes, esters, and alcohols.

According to the fourth criterion, new regulations focus on building products or consumer products used inside the buildings. The fifth and sixth criteria are applied to indoor chemicals having similar uses or chemical structures with substances mentioned by the established IAQ guidelines. As a result, IAQ guidelines for 13 chemicals were established (Table 1) on the basis of scientific discussions in the CIAP.

The guidelines regarding acceptable values for indoor air concentrations of these chemicals were established on the basis of the studies measuring chronic toxicity over long-term exposure. However, one exception is formaldehyde, which was given a 30-min average value based on toxicity over short-term exposure. This is because the main objective of formaldehyde exposure is to avoid short-term irritation and consecutively repeated irritation. Toxicologically based guideline values are derived from the toxic effects and dose-response relationships for critical toxic endpoints based on the recommendations of the WHO (WHO, 1999).

For substances with a threshold in the dose-response relationship, critical effect levels as a point of departure (POD), such as lowest-observed adverse effect level (LOAEL) or no-observed adverse effect level (NOAEL), are determined. Then, various assessment and extrapolation factors are applied to the POD, as shown in Table 1. For substances with no threshold in the dose-response relationship, such as genotoxic carcinogens, unit risk is calculated. In general, an air concentration corresponding to a lifetime excess cancer risk of 1/100,000 (10^{-5}) is used as the guideline in Japan (Kawamoto et al., 2011).

In these processes, when critical effect levels of inhalation exposure are not identified, critical effect levels derived from oral exposure studies are used. This is based on the assumption that chemicals that cause adverse health effects after exposure by ingestion cause health effects at the same target site after intake into the body by inhalation (and *vice versa*) (OEHHA, 2005). This assumption depends on the critical effects of the chemical. This assumption is acceptable for the systemic effect but not for the local effect, such as respiratory versus digestive. To make this conversion, a reference human body weight of 50 kg and a reference human respiration rate/day of 15 m^3 are used.

Table 1
Guidelines for indoor air quality.

Substances	Point of departure[a]	Critical toxic endpoint	Assessment and extrapolation factor[b]	Guideline value (μg/m^3)	Date of establishment	Reference
Formaldehyde	Inhalation 0.1	Nose and throat irritation in humans	Not applied	100	1997.6.13	WHO Europe (1996)
Toluene	Inhalation LOAEL 332	CNS and reproductive effects in humans	ACE: 4.2, UF1: 10, UF4: 10, Potential effects on the developing CNS: 3	260	2000.6.26	Foo et al., (1990), 1993, Ng et al., (1992)
Xylene	Inhalation LOAEL 61	CNS effects in humans	UF1: 10, UF4: 10, Lack of chronic neurological effects: 3	200 (870 previous value)	2019.1.17 revision (2000.6.26 initial)	Uchida et al. (1993)
1,4-Dichlorobenzene	Oral NOAEL 10	Liver and kidney effects in dogs	ACE: 1.4, UF3: 10, UF4: 10, AIE: 0.3	240	2000.6.26	Naylor and Stout (1996)
Ethylbenzene	Inhalation NOEL 2150	Liver and kidney effects in mice and rats	ACE: 5.6, UF 3: 10, UF4: 10	3800	2000.12.15	NTP (1992)
Styrene	Inhalation LOAEL 1260	Brain and kidney effects in rats	ACE: 5.6, UF1: 10, UF 3: 10, UF4: 10	220	2000.12.15	Savolainen and Pfäffli (1977), Vainio et al., (1979)
Chlorpyrifos	Oral LOAEL 0.3	Neurological effects in maternal rats and morphological effects of brain in the neonatal infants	UF1: 10, UF 3: 10, UF4: 10, AIE: 0.3, Additional UF for brain effects in children: 10	1 for adults, 0.1 for children	2000.12.15	Hoberman (1998), USEPA 2000a,b
Di(n-butyl) phthalate	Oral LOAEL 2.5	Reproductive and developmental effects in rats	UF1:5, UF3:10, UF4: 10, AIE: 0.3	17 (220 previous value)	2019.1.17 revision (2000.12.15 initial)	Lee et al. (2004)
Tetradecan	Oral NOAEL 100	Liver effects in rats	UF2: 10, UF3: 10, UF4: 10, AIE: 0.3	330	2001.7.5	TPHCW (1997)
Di(2-ethylhexyl) phthalate	Oral NOAEL 3	Reproductive and developmental effects in rats	UF3:10, UF4: 10, AIE: 0.3	100 (120 previous value)	2019.1.17 revision (2001.7.5 initial)	Christiansen et al. (2010)
Diazinon	Oral LOAEL 0.026	Neurological effects in rats	UF1: 3, UF3: 10, UF4: 10, AIE: 0.3	0.29	2001.7.5	USEPA (2000c)
Acetaldehyde	Inhalation NOEL 270	Effects on olfactory epithelium in rats	ACE: 5.6, UF2 combined with possible cancer effect: 10, UF3: 10, UF4: 10	48	2002.1.22	Appelman et al. (1986)
Fenobucarb	Oral NOEL 4.1	Neurological effects in rats	UF3: 10, UF4: 10, Difference of absorption rate in inhalation exposure: 4 AIE: 0.3	33	2002.1.22	Mitsubishi Chemical Corporation (1990)
TVOC	As low as reasonably achievable from nationwide survey of VOCs			400 tentative target value	2000.12.15	MHW (1999)

[a] Inhalation (mg/m^3). Oral (mg/kg/day).

[b] UF1, LOAEL to NOAEL extrapolation; UF2, Extrapolation across durations; UF3, Interspecies extrapolation; UF4, Intraspecies extrapolation. Abbreviations: ACE, adjusting from discontinuous exposure to continuous exposure (24 h per day, 7 days per week); AIE, adjusting from oral intake to inhalation exposure (human body weight of 50 kg and human respiration rate/day of 15 m^3); CNS, central nervous system; LOAEL, lowest-observed adverse effect level; NOEL, no-observed effect level; NOAEL, no-observed adverse effect level; UF, uncertainty factor; TVOC, total volatile organic compounds; VOCs, volatile organic compounds.

2.2.3. Non-toxicologically based advisable value for TVOC

The individual guideline values for indoor air chemicals is based on toxicological data. However, a large number of indoor air chemicals have been detected (MHW, 1999; MHLW, 2000a), and establishing individual guideline values would require a great amount of time. Furthermore, the health risks from potentially hazardous chemicals whose guideline values are not yet established may increase in the future. Hence, the CIAP adopted the TVOC approach as an important indicator to limit the indoor air pollution (MHLW, 2000b). The TVOC value indicates the total amount of individual VOCs as derived from a gas chromatography/mass spectrometry (GC/MS) curve from n-hexane to n-hexadecane (JRC, 1997).

Originally, the CIAP did not have sufficient reliable scientific knowledge to establish the guideline value of TVOC based on the available toxicological data. Consequently, the CIAP recommended a tentative target value of 400 μg/m^3 from the median value calculated using the results of a nationwide field survey on VOCs (MHW, 1999), based on the concept of "as low as reasonably achievable" (ALARA) (MHLW, 2000b). The association between TVOC concentration and indoor health effects or complaints, like building-related symptoms, sensory irritations, or chemical intolerance, is not straightforward (Wolkoff and Nielsen, 2001). Hence, the TVOC target value should be used as an indicator for IAQ, independently of individual VOC guideline values.

2.3. Effect of setting IAQ guidelines

Since IAQ guidelines were established from 1997 to 2002, the mean indoor air concentrations of formaldehyde, toluene, xylene, and ethylbenzene in the nationwide survey on housing notably decreased from 2000 to 2005; i.e., from 89.6 to 29.5 μg/m^3 for formaldehyde, 154.2 to 11.3 μg/m^3 for toluene, 26.0 to 4.3 μg/m^3 for xylene, and 43.4 to 4.3 μg/m^3 for ethylbenzene. However, this tendency was not observed for acetaldehyde from 2002 to 2005 (30.6–30.6 μg/m^3) (Osawa and Hayashi, 2009).

The main emission source of formaldehyde is adhesives for wood-based materials, and shipments of formaldehyde-based adhesives decreased since setting the guideline. The main emission sources of toluene, xylene, and ethylbenzene are solvent-based paints, and shipments of such paints have also decreased (Azuma et al., 2008). The efforts of the related industries resulted in those reductions. However, there are numerous sources of acetaldehyde emissions in indoor environments, such as construction lumber, incomplete combustion in appliances, environmental tobacco smoke, drinking alcohol, and fragranced consumer products (Yamashita et al., 2010). Generation from these sources largely depends on occupant lifestyle, and secondary acetaldehyde sources can make it difficult to reduce indoor air concentration. 1,4-Dichlorobenzene is used for indoor household insect repellents, the usage of which in buildings depends on the lifestyle of the occupants. The health risk of 1,4-dichlorobenzene remained at a high level in the results of a nationwide survey of housing conducted from 2012 to 2014 (Azuma et al., 2016). Thus, additional approaches for the risk management of substances that depend on the lifestyles of building occupants are required.

2.4. New scheme for developing IAQ guidelines

The CIAP proposed a new scheme for developing IAQ guidelines based on health risk assessments, including the priority of the indoor air chemicals for which guideline values should be established (Fig. 1) (MHLW, 2013a). According to the new scheme, preliminary exposure assessments are initiated on the basis of the exposure data compiled from nationwide field surveys, emissions from household products, or epidemiological studies. Subsequently, preliminary risk assessment is conducted using data from the exposure assessments and existing hazard data. Then, priority-setting for developing IAQ guidelines is carried out based on the risk levels. To obtain the POD for hazard assessment, the latest documents and reports published by international and national agencies are reviewed, and toxicological or epidemiological studies are searched using databases of medical and scientific literature. The most sensitive toxic endpoint is selected to minimize the risks associated with chemical exposure.

The National Institute of Health Sciences, which is a major organization within the MHLW, conducted nationwide field surveys on the indoor air concentrations of chemicals in housing from 2011 to 2013 (MHLW, 2013b, 2013c, 2014). Based on these surveys, the CIAP conducted preliminary exposure and risk assessments for chemicals detected in the nationwide field survey (MHLW, 2016). Table 2 shows a summary of the preliminary exposure and health risk assessments that are combined with the results of those nationwide field surveys.

In order to help risk assessors assist risk managers in making judgments on the overall level of concern and in prioritization of competing hazards, several bodies have proposed the use of margin of exposure (MOE) for comparative risk assessment (Omenn et al., 1997; WHO, 1999; WHO, 2006). MOE reflects the ratio between a level associated with toxic effects and the actual level of exposure in a particular situation (Omenn et al., 1997). The CIAP adopted the MOE approach for characterizing health risks from exposure to indoor air chemicals measured in the nationwide field surveys. The results show that the risk levels for 2-ethyl-1-hexanol, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (TMPD-DIB), and 2,2,4-trimethyl-1,3-pentanediol monoisobutyrate (TMPD-MIB) indicate a "potential for health risk" because of their low MOEs. In the detailed risk assessment, the major uses and emission sources of chemicals that the IAQ guidelines were previously established as well as further detailed data on exposure, are considered for the management of novel target chemicals for developing IAQ guidelines. Furthermore, currently available methods of measurement and the confirmation of appropriate political effects are also considered. The IAQ guidelines for these three chemicals are under development by the CIAP.

3. Future issues for approaches to indoor chemicals

Mechanisms for single route of exposure via inhalation and exposure to a single chemical are generally considered in the existing IAQ guidelines. However, there are multi-route and multi-media mechanisms of exposure for specific indoor chemicals. Exposure to a single chemical through multiple routes and from multiple sources is defined as an aggregate exposure (Kienzler et al., 2016; Meek et al., 2011). In addition, when there are multiple exposures to indoor chemicals, combined health effects are often a cause for concern. An instance of exposure to multiple chemicals is defined as a combined exposure. The aggregate and combined exposures from mixtures occur upon exposure to various chemicals including VOCs, SVOCs, and particulates. In this section, we discuss these two future issues regarding risk assessment approaches for indoor chemical exposure. In particular, we focused on indoor exposure to SVOCs as examples of aggregate exposures.

3.1. Multi-route and multi-media exposure (i.e., aggregate exposure) to SVOCs

SVOCs include a vast array of plasticizers, flame retardants, pesticides, biocides, preservatives, sealants, surfactants, waxes, and polishes. They are widely used as additives in many building materials, furniture products, carpets, and consumer products (Lucattini et al., 2018; Weschler and Nazaroff, 2008). Vapor pressures of these SVOCs between 10^{-14} and 10^{-4} atm (10^{-9} to 10 Pa) have been proposed. Many phthalates, perfluorinated compounds, organophosphate compounds, chlorinated compounds, brominated compounds, and siloxanes are classified as SVOCs. Furthermore, SVOCs are found in both the gas and condensed phases and redistribute from their original sources over time to indoor air, indoor dust, and other indoor surfaces (Weschler and

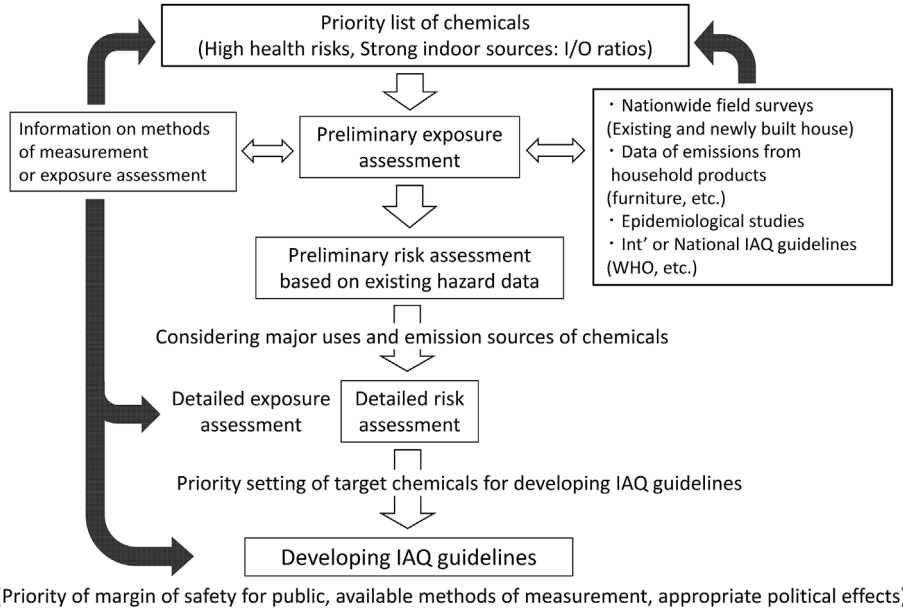

Fig. 1. Scheme for developing indoor air quality guidelines. Abbreviations: IAQ, indoor air quality; I/O, indoor/outdoor; Int', international; WHO, world health organization.

Nazaroff, 2008).

Phthalates are ubiquitous chemicals found in many consumer products and plasticizers. They impart flexibility and durability to resins such as polyvinyl chloride (Huber et al., 1996). Phthalates can be released into the environment through leaching, evaporation, migration, and abrasion. Due to their widespread use, the general population is constantly exposed to phthalates in everyday life through ingestion, inhalation, and skin absorption from indoor air, indoor suspended particles, indoor dust, and contaminated food or drinking water (Bekö et al., 2013; Hauser and Calafat, 2005; Koch et al., 2013; Weschler and Nazaroff, 2008; Wormuth et al., 2006). This multi-route and multimedia exposure is defined as aggregate exposure (Kienzler et al., 2016). These situations and relationships are illustrated in Fig. 2.

In study on Danish children, Bekö et al. (2013) reported that exposures to air and dust in the indoor environment for diethyl phthalate (DEP), di(n-butyl) phthalate (DnBP), and di(isobutyl) phthalate (DiBP), which have higher vapor pressures, accounted for approximately 100%, 15%, and 50% of the total intake, respectively, with dermal absorption from the gas-phase being the major exposure pathway. More than 90% of the total intake of butyl benzyl phthalate (BBzP) and di(2-ethylhexyl) phthalate (DEHP), which have lower vapor pressures, came from sources other than indoor air and dust. Guo and Kannan (2011) reported that dietary intake and dermal absorption were important but mutually-interfering sources of DEP exposure in the USA, whereas dietary intake was the main source of BBzP exposure (> 58%) and DEHP exposure (> 86%). Inhalation, dermal absorption, and dietary intake were mutually-interfering main sources of DiBP.

To reduce the health risks due to chemical pollutants via such these multi-route exposures, risk management based on estimates of the total body burden of the pollutants and the relative contributions (i.e., allocation) of these exposures to the total body burden should be performed. Therefore, some consideration of the proportion of the acceptable daily intake (ADI) or tolerable daily intake (TDI) attributed to different exposure sources is needed for developing guideline values and risk management strategies. This approach ensures that total daily intake from all exposure sources does not exceed the ADI or TDI.

In Japan, this approach was used in the development of ambient environmental quality standard for dioxins. Over 90% of human daily intake of dioxins results from the consumption of food containing dioxins. The major environmental sources of dioxins are emissions from combustion, waste incineration, and production process of industrial chemicals. Aerial transport of these emissions is the primary pathway by which dioxins enter the terrestrial environment and food chain (WHO, 2000). Therefore, setting a standard for dioxins in ambient air was required. Before developing an ambient environmental quality standard for dioxins, the Environment Agency (EA, currently the Ministry of the Environment) and the Ministry of Health and Welfare (MHW, currently the MHLW) set a TDI of 4 pg-TEQ/kg/day for dioxins based on toxicological data from animals (EA and MHW, 1999). Then, in 1999, the EA established an annual average ambient environmental quality standard of 0.6 pg-TEQ/m^3 for dioxins based on the TDI along with the assumptions that i) the internal absorption rate of dioxins is 50% from food and 85% from ambient air; and ii) the allocation rate of ambient air is 5–15% of the total intake of dioxins in general populations (Kawamoto et al., 2011).

This allocation approach has also been reported in performing risk assessments for drinking-water contaminants (Krishnan and Carrier, 2008, 2013) and used in establishing WHO guidelines for drinking-water quality (WHO, 2017). For instance, in the drinking-water quality guideline for trichloroethylene, the WHO estimated 50% of the TDI as being the allocation factor for drinking water in the total body burden of trichloroethylene, with the remaining 50% coming from inhalation of air and food intake. In the case of chloroform, the WHO estimated 75% of TDI as the allocation factor for ingestion of drinking water, with the remaining 25% coming from inhalation of indoor air (largely due to volatilization from drinking-water) and dermal exposure during showering or bathing.

This consideration of allocations for exposure pathways is an important approach to reducing the total body burden for substances with multiple exposure pathways. However, this approach should be carefully applied only in cases where the target organ and the critical endpoint for inhalation, ingestion, and dermal exposures are the same. Furthermore, it should be based on detailed data for the external exposure allocation and internal absorption, distribution, metabolism, and excretion. This approach is generally applied when the TDI, ADI, standard, or guideline is derived using toxicological data from animals, because the exposure route in the experimental animal study is usually properly controlled. Thus, this approach is not generally applied when these values are derived from human epidemiological data. This is because instances of all exposure during daily activities, including

Table 2
Preliminary exposure assessment and health risk assessment.

Substances	Emission source	Season	*n*	Construction	Maximum indoor air concentration (μg/m^3)	Critical toxic endpoint	Human critical effect level[b] (mg/m^3)	Reference	MOE[a]
2-Ethyl-1-hexanol	hydrolytic degradation of DEHP	2013 summer	93	Existing	133	Eye irritation in humans	NOAEL 8[c] (NOAEL 283)	Kiesswetter et al., (2005) (Klimisch et al., 1998)	60 (2125)
TMPD-MIB	Water-based paint	2012 winter	39	New-built	837	Liver effects in rats	NOAEL 244	O'Donoghue (1984)	292
TMPD-DIB	Water-based paint	2012 winter	39	New-built	661	Liver and renal effects in rats	NOAEL 100	MHW (1993)	151
Ethyl Acetate	Adhesives or paints	2012 summer	93	Existing	203	Degeneration of olfactory epithelium in rats	LOAEL 557	Christoph et al. (2003)	2744
Butyl Acetate	Adhesives or paints	2012 winter	39	New-built	664	Inhibited body weight and neurological effects in rats	NOAEL 1061	David et al. (2001)	1598
PGME	Paints	2012 winter	111	Existing	135	Liver effects in rats	NOAEL 488	Landry et al. (1983)	3615
MMB	Adhesives	2013 summer	93	Existing	93	Liver and renal effects in rats	LOEL 142	OHSC (1976)	1530
DEGME	Paints	2012 winter	39	New-built	337	Overall findings	NOAEL 469	Miller et al. (1985)	1391
DEGEE	Paints	2012 winter	39	New-built	192	Respiratory irritation in rats	NOAEL 40	Hardy et al. (1977)	207
PGMEA	Adhesives or paints	2012 winter	39	New-built	253	Renal effects in rats	NOAEL 716	Miller et al. (1984)	2831
MIBK	Adhesives or paints	2012 winter	39	New-built	151	Renal effects in rats	LOEL 818	Stout et al. (2008)	5417

[a] MOE is calculated from dividing human critical effect level by maximum indoor air concentration.
[b] Human critical effect level is determined from effect level observed in reference and if needed adjusting from discontinuous exposure to continuous exposure (24 h per day, 7 days per week) and adjusting from animal body burden to human equivalent exposure concentration.
[c] NOAEL was revised after discussion on the preliminary risk assessment. Parentheses were values and reference in the preliminary risk assessment. Abbreviations: TMPD-MIB, 2,2,4-trimethyl-1,3-pentanediol monoisobutyrate; TMPD-DIB, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate; PGME, Propylene Glycol Monomethyl Ether; MMB, 3-Methoxy-3-methylbutanol; DEGME, Diethylene Glycol Methyl Ether; DEGEE, Diethylene Glycol Ethyl Ether; PGMEA, Propylene Glycol Monomethyl Ether Acetate; MIBK, Methyl Isobutyl Ketone; DEHP, Di-2-ethylhexyl phthalate; LOEL, lowest-observed effect level; LOAEL, lowest-observed adverse effect level; NOAEL, no-observed adverse effect level; MOE, margin of exposure.

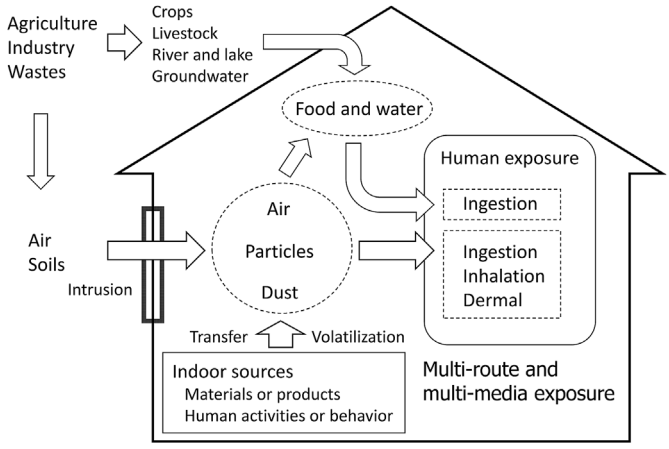

Fig. 2. Pathways to human exposures from sources of SVOCs.

inhalation, ingestion, and dermal exposures, could be spontaneously included in the epidemiological study. However, this approach is needed for epidemiological data from regions where the background levels of exposure are very different. For instance, high-exposure regions and low-exposure regions may have different background levels of food contaminants, affecting the IAQ guidelines for aggregate exposure.

3.2. Multiple exposures to indoor air chemicals (i.e., combined exposure) and the combined health effects

Multiple low-level indoor chemical pollutants are found in indoor environments. Although the indoor air concentration of each pollutant may be low, when many of these pollutants exist collectively in an indoor environment, a greater combined health risk (i.e., additive effects) may be created (Azuma et al., 2016). This situation is defined as combined exposure (Kienzler et al., 2016). The effects of environmental pollutants have been mainly focused on health outcomes from single exposures to single substances, and other multiple risks have been often attributed to confounding in epidemiological studies. However, several epidemiological studies have revealed significant increases in odds ratios for indoor air concentrations that do not exceed IAQ guidelines or reference concentrations (Arif and Shah, 2007; Azuma et al., 2018; Bentayeb et al., 2013; Billionnet et al., 2011; Takigawa et al., 2010; Takigawa et al., 2012). Thus, an approach for evaluating the combined risks of chemical pollutants with similar health effects and future strategies for limiting the total health risk due to multiple low-level indoor chemical pollutants is required.

There are four types of combined effect or interaction: dose addition, response addition, synergism, and antagonism. Since the 1990s, numerous evaluations of combined exposures to mixtures of substances (i.e., food additives, pesticides, veterinary drugs, and contaminants) have been especially undertaken by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA), the Joint FAO/WHO Meeting on Pesticides Residues (JMPR), and the Scientific Committee for Food of the European Commission (subsequently the European Food Safety Authority (EFSA)). Accordingly, group ADI or TDI values have been recommended for several chemical groups as a means to limit their overall intake. For this procedure to be feasible, the substances should have a similar mode of action and a similar range of toxic potency.

An approach that takes account of dose additivity is the toxic equivalency factor (TEF) approach, which scales the exposure for each component of a mixture relative to the potency of an index chemical, such as for dioxins and dioxin-like chemicals (WHO, 2009). Recently, the Panel on Food Contact Materials, Enzymes, and Processing Aids (CEP) of the EFSA has proposed a group TDI of 50 μg/kg bw per day for four phthalates, i.e., di-butyl phthalate (DBP), BBzP, DEHP, and diisononyl phthalate (DINP). The group TDI is based on a plausible common mode of action for the anti-androgenic effect underlying the male reproductive toxicities of these four phthalates (EFSA, 2019a).

The German Committee on Indoor Guide Values (or Ausschuss für Innenraumrichtwerte (AIR)) of the German Environment Agency has established toxicologically based indoor air guide values for over 50 substances or substance groups (Fromme et al., 2019). The AIR defined toluene, ethylbenzene, and xylenes as comprising a group because all C_7–C_8 alkylbenzenes have similar neurotoxic effects. The AIR proposed that, in order to achieve a total evaluation, the ratios of concentrations and guideline values of each compound should be summated, providing a whole sum of risks. The total guideline values are regarded as being complied with if the corresponding sum falls below 1 (AIR, 2016).

This approach also takes into account dose additivity. The concept of dose addition has been the most widely used to determine the common toxic effects of combinations of chemicals (Boobis et al., 2011; EFSA, 2019b; Kortenkamp and Faust, 2018; Meek et al., 2011). This is one approach to assessing the combined risks of pollutants with similar toxicological effects.

Clearly, risks from combined exposure to multiple chemicals as well as methodologies to assess those risks have been discussed, and methodologies and guidance for assessing risks from combined exposure to multiple chemicals have been developed for different regulatory sectors. However, a harmonized, consistent approach for performing mixture risk assessments and management across different regulatory sectors is still lacking (Bopp et al., 2019; Kienzler et al., 2016; Kortenkamp and Faust, 2018; OECD, 2018; Rotter et al., 2018). In particular, related research on indoor chemicals is rare. Given the diversity of possible combinations of chemicals and the diversity of possible approaches, such as those based on use or release, chemical structures, or similar toxicological effects, the application of different approaches and methods can depend on the assessment context and problem formulation. Thus, further research into harmonized approaches to risk assessment of combined exposures to multiple chemicals in indoor environments is needed.

Although health risk assessments based on the measurement of specific pollutant air concentrations have been applied as a means to assess environmental health risks so far, novel approaches to health risk assessment using environmental biomarkers (e.g., sensory irritation markers, central nervous system effect markers, oxidative stress markers, or mutagenic markers, which directly represent health stress due to environmental factors) to evaluate biological and health effects are required to replace existing health risk assessment methods. This approach will prevent negative health effects caused by combined exposure to multiple low-level pollutants and/or exposure to alternative chemicals that potentially pose the risk of impairing human health.

4. Concluding remarks and future perspectives

IAQ guidelines have been established and continue to be refined based on the fundamental concept that, in order to protect public health, unnecessary exposure to indoor chemicals should be minimized and chemicals should be safely and appropriately used so that they have no adverse effects on human health. The types and concentrations of indoor chemicals have shifted over time due to lifestyle changes and the development of novel household products and building materials. Therefore, the continued monitoring of indoor chemicals and the development of IAQ guidelines for substances that present potentially high health risks are essential for ensuring public health. Moreover, in indoor environments, there are multiple media by which humans come in contact with indoor chemicals and multiple exposure pathways that can affect human health, particularly for SVOCs. Furthermore, combined exposure to multiple low-level chemical pollutants occurs in indoor environments. Therefore, development of an integrated multi-pollutant and multicompartmental approach for limiting aggregate and combined exposures is essential in order to determine the extent of

threats to public health posed by indoor chemicals. Developing approaches to assess health risks using environmental biomarkers that directly represent health stress due to environmental factors will be required as the future approach to prevent negative health effects caused by combined exposures to multiple low-level pollutants.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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