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Benzene Exposure in Workers From a Waste Oil Regeneration Plant During Ordinary Activities by Air and Biological Monitoring

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Abstract

Background: *In the regeneration of waste oil, a strategical technological process for the European Union circular economy action plan, exhausted oils are regenerated to produce high performing oil bases. Aim of this work was to assess the exposure to benzene in plant workers during ordinary activities.* **Methods:** *59 workers, potentially exposed to benzene, and 9 administrative workers from an Italian plant were monitored for the whole work shift with personal air samplers; urinary benzene (BEN-U) and S-phenyl mercapturic acid (SPMA) were measured by mass spectrometry methods in end-shift urine samples. Different job tasks were identified among workers.* **Results:** *Median (minimum-maximum) airborne exposures to benzene were <0.9 (<0.9-6.3) and <0.9 (<0.9-0.9) µg/m^3, BEN-U and SPMA levels were 0.094 (<0.015-3.095) µg/L and 0.15 (<0.10-9.67) µg/g crt and 0.086 (0.034-0.712) µg/L and <0.10 (<0.10-3.19) µg/g creatinine in workers and administrative workers, respectively. No differences were found among job tasks and between workers and administrative workers, while higher levels were found in smokers than in non-smokers. For all job tasks, the exposure to benzene was always below occupational limit values.* **Conclusions:** *This study has investigated for the first time the exposure to benzene of workers employed in the re-refining of exhaust oil. The results showed that normal production activities in regenerating used oils do not pose a risk of exposure to benzene in workers.*

1. Introduction

Lubricating oils are liquid mixtures used in several industrial sectors for the lubrication of mechanical parts, to prevent metal-to-metal contact, remove contaminants, cool machine surfaces, remove wear debris, and transfer power.

The main component of lubricating oil is represented by the base oil (53-99% in volume), which can be produced either by chemical synthesis (synthetic oils, such as polyalphaolefins) or by distillation from crude oil (mineral oils). To meet the desired characteristics for specific uses, several additives are added to the base oil (generally present from 1 to 30% by volume), such as antioxidants, anti-wear agents, detergents, and dispersants [1].

Due to mechanical stress, the possibly high operating temperatures and pressure, and the contact

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with engine parts, lubricating oils undergo additive depletion and dilution with several contaminants, including water, chlorides, antifreeze, fuel, light hydrocarbons, metals, solids, sulphur, monoaromatic and polycyclic aromatic hydrocarbons [1, 2]. Waste oils are considered hazardous waste with dangerous properties and are managed in Europe according to the Waste Framework Directive 2008/98/EC, following the repeal of the Waste Oils Directive 74/439/EEC [3]. In particular, the directive 2008/98/EC stipulates that waste oil must be treated, prioritising regeneration or alternatively to other recycling operations (i.e. preparation of fuels, energy recovery, and hazardous waste incineration) delivering an equivalent or a better overall environmental outcome than regeneration. Besides, as recently underlined by the European Union Green Deal and Circular Economy Action Plan [4], oil regeneration has become a key technological process for Europe.

Various industrial processes may be used for waste oil regeneration (also called re-refining), including high-pressure hydrogenation, distillation, acid/clay process, and extraction with compressed propane. Distillation processes, in particular, may use different combinations of vacuum and atmospheric pressure distillation [5].

A national consortium for the management, collection, and treatment of mineral waste oils (CONOU) operates in Italy, with the main tasks of ensuring and promoting waste oil collection throughout the national territory and ensuring that the waste oils collected are sent to the most appropriate treatment and primarily to re-refining for the production of base oils. About 93% of the collectable waste oil was collected in 2017 in Italy, while the amount of regenerated oil placed on the market in 2018 was about 400,000 tonnes, 46.7% of lubricant oils. Up to 100% of the waste oil collected in Italy is regenerated to produce re-refined base oil, while only minimal amounts go to energy recovery or incineration, making Italy one of the leading countries in Europe in this field [5].

Given the strategical role in de-carbonising the economy and minimising the generation of waste and pollution [6], oil regeneration activities are acknowledged among the so-called green jobs. However, workers in the green industries may face hazards that are commonly known in classical workplaces [7]. Given the hazardous properties of waste oils, workers employed in plants dealing with its regeneration may be exposed to several chemical contaminants. Among these, benzene is of particular relevance for its toxicological properties.

Benzene is a known carcinogen to humans (group1) [8] and it is a category 1A (H350) carcinogen according to the European Commission (EU) [9]. To protect workers' health from the occupational exposure to benzene, the EU revised the former limit value of 3300 μg/m^3 (1 ppm) [10] and set a revised limit value of 660 μg/m^3 (0.2 ppm) to enter in force on 5 April 2026 [11]. As a transitional measure, the limit value of 1 ppm should continue to apply until 5 April 2024 and a transitional limit value of 0.5 ppm (1650 μg/m^3) should apply from 5 April 2024 until 5 April 2026 [11]. For the biological monitoring of benzene, the measurement of S-phenylmercapturic acid (SPMA) in end-of-shift samples is recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) [12], while the measurement of urinary benzene (BEN-U) has also been proposed as a sensitive and specific marker of exposure to benzene by the German Deutsche Forschungsgemeinschaft (DGF) and by the European Chemicals Agency (ECHA] [13, 14].

The aim of this study was to assess the exposure to benzene in workers employed in a regeneration plant in Italy during normal activities by personal exposure air sampling and biological monitoring of SPMA and BEN-U.

2. Methods

2.1. Study Population

The study was performed from June 15 to 28, 2017, in a plant for the regeneration of exhaust oil in the province of Lodi (Italy). The plant's total treatment capacity is around 200,000 tonnes per year, and it produces mainly regenerated lube bases, diesel oil, and a mixture for applications in bituminous membranes.

The study population consisted of 68 healthy adults, among which 59 were plant workers (here referred to as "workers"), and 9 were workers from the administrative staff of the same plant (administrative workers). Based on job tasks, different working units were identified, including exhaust oil receiving (REC), remote and on-site plant control (PLANT), plant maintenance supervising (MAN), exhaust and regenerated oil quality controls (LAB), and regenerated oil storage and delivery (DEL). Workers involved in the remote and on-site plant control worked in 8-h shifts throughout the 24 hours (day: 8:00 a.m.-4:00 p.m., afternoon: 4:00 p.m.-24:00 p.m.; night: 24:00 p.m.-8:00 a.m.), while the other workers and the administrative staff worked 8-h day shifts (typically 7:00 a.m.-3:00 p.m. or 8:00 a.m.-4:00 p.m.). Samplings were performed in all shifts, including 1 afternoon-, 2 night-, and 4 day-shifts. All workers wore protective equipment (overall, gloves, helmet, and safety shoes). Workwear is changed weekly or at the worker's convenience, if necessary, and a laundry service exists.

Data regarding personal characteristics, health status, active and environmental tobacco smoke, diet, lifestyle (commuting time and means of transport; car refuelling, use of solvents, dyes or paints in the spear time, and biomass burning in the previous 24 hours), and residential characteristics (rural, urban peripheral, or urban area, presence of industrial sites near residence, intensity of traffic at residence, presence of a car garage linked to house) were collected by a questionnaire administered by trained interviewers.

All the operations related to the sampling during the monitoring campaign were carried out in a clean room located in the plant's administration building.

2.2. Personal Exposure to Benzene

Individual personal exposure to airborne benzene (BEN-A) was monitored for the whole shift. Air was sampled using the passive sampler Radiello equipped with a 35-50 mesh charcoal cartridge (Merck, Milano, Italy). Workers wore the sampler on their upper chest, near their respiratory zone. At the end of the sampling period, the cartridge was sealed in the appropriate glass tube and stored in a clean box at room temperature until analysis. Cartridges were analysed within 30 days of collection, according to the manufacturer's instructions.

Airborne benzene was measured by gas chromatography coupled to mass spectrometry (GC-MS) [15]. Quantification limit (LOQ) was 16 μg/L. Considering the average sampling time and the uptake rate of airborne benzene, this concentration was estimated to correspond to airborne levels of 0.9 $\mu g/m^3$.

2.3. Urine Samples Collection and Analysis

Urine spot samples were collected in disposable polyurethane tubes at the end of the shift on the same day of the air sampling. A disposable syringe was used to immediately place a 7 ml aliquot in a pre-sealed glass storage vial for the determination of urinary benzene, while a 10 ml aliquot was poured in a polyethylene tube for the determination of SPMA, cotinine, and creatinine (crt). Samples were immediately stored at -20°C and delivered frozen to the laboratory at the end of the survey. All aliquots were kept at - 20°C and analysed, according to biomarkers' stability, within 60 days. Samples were coded and then handled without knowledge of their origin.

Urinary benzene (BEN-U) was determined by headspace solid-phase microextraction (HS-SPME) followed by GC-MS analysis according to according to a published method [16]. The LOQ for BEN-U was 10 ng/L. The urinary SPMA was determined by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method [17]. The LOQ was 0.01 μg/L. Urinary cotinine (COT-U), a biomarker of tobacco smoking, was measured by LC-MS/MS. The LOQ was 0.1 μg/L [18]. Subjects with COT-U below 30 μg/L were classified as 'nonsmokers', while subjects with COT-U equal to or above 30 μg/L were classified as 'smokers' [19]. Jaffe's colourimetric method determined urinary creatinine (crt) [20]. No criteria of acceptability based on urine dilution were applied.

For each analyte, calibration curves covering the expected range of concentrations were used. Two concentrations from the low and middle of the calibration curve for each analyte were used as

internal quality control (QC) samples. A calibration curve was run with every set of unknown samples, so the typical analytical sequence was defined as a calibration curve followed by the unknown samples (n=20) prepared and analysed along with two duplicates (unknown samples randomly chosen) and two QC samples. Moreover, the quality control of the method to quantify BEN-U, SPMA, and COT-U is guaranteed by the successful participation twice a year to the German External Quality Assessment Scheme (G-EQUAS) for analyses in biological materials [21].

2.4. Statistical Analysis

Statistical analysis was performed using the SPSS 28.0 package for Windows (SPSS Statistics, IBM Italia). Descriptive analyses were used to obtain the median and ranges of ambient and biological analytes. A value corresponding to one-half of the quantification limit was assigned to measurements below analytical quantification. Additional statistical analyses were performed on decimal log-transformed to ensure normal distribution. Student's t-test or analysis of variance was applied to compare two or more groups of independent samples, Pearson's correlations were used to test the associations between variables, and the chi-square test was used to compare the percentage distribution of positive values among groups. The raw values calculated from the integration of analytical peaks were used unchanged instead of applying substitution methods (e.g., fractions of the quantification limit) to avoid substantial bias by substitution [22]. The chi-square test was used for analytes with less than 50% of the data above the LOQ.

3. Results

3.1. Study Population

Table 1 reports the main characteristics of the investigated population. Subjects were predominately males, with only 4 female workers (2 among the administrative staff and 2 among the LAB unit). Of 59 plant workers, 4 were from the REC, 6 from the DEL, 6 from the LAB, 8 from the MAN, and 35 from the PLANT unit. The mean age (46 vs. 46 years) and BMI (26.2 vs. 26.1 kg/m^2) were similar between administrative and plant workers. According to the questionnaire, subjects classified themselves as non-smokers (67 and 54% among administrative and plant workers, respectively), traditional cigarette smokers (33% and 32%), or e-cig smokers (12%, all plant workers). Median COT-U levels in administrative and plant workers were 0.38 and 0.35 μg/L in non-smokers, 2023 and 1554 μg/L in smokers, and 1530 μg/L in e-cig smokers, respectively. The COT-U measurements were consistent with the smoking status self-classification. In smokers, the mean number of cigarettes/day was 12 and 13, the mean number of cigarettes smoked before the shift was 1 and 2, while the mean number of cigarettes smoked during the shift was 4 and 5, in administrative and plant workers respectively.

3.2. Personal Exposure to Benzene

Table 2 reports the results of the personal exposure to benzene. Samples were available for 67% and 89% of administrative and plant workers, respectively. The median sampling time was 402 minutes (min 222, max 480 minutes).

Air benzene was detectable only in 17 and 44% of samples, with median (minimum-maximum) levels <0.9 (<0.9-0.9) μg/m^3 and <0.9 (<0.9-6.3) μg/m^3 in administrative and plant workers. One subject from the PLANT unit, with BEN-A > 30 μg/m^3, was excluded from the statistical elaboration, as this value was considered an outlier due to probable sample contamination. No significant difference was found (p-value for χ-test= 0.179) between plant workers and administrative workers and comparing the different job tasks among plant workers (p-value for χ-test= 0.102).

Figure 1 shows the distribution of benzene exposure in workers and administrative staff in comparison with the EU occupational limit values for benzene (3300, 1650, and 660 μg/m^3) and the European air quality target value as a mean calendar year limit (5 μg/m^3). All subjects had benzene exposure below these limits, but one subject from the REC unit had benzene exposures above 5 μg/m^3 (6.3 μg/m^3).

Table 1. Summary of selected characteristics in all subjects and in subjects stratified according to their job.

		Amministrative workers	Plant workers	Plant Units				
				REC	DEL	LAB	MAN	PLANT
Number of subjects, N		9	59	4	6	6	8	35
Male sex, N (%)		7 (78)	57 (97)	4 (100)	6 (100)	4 (67)	8 (100)	35 (100)
Age, mean (min-max) year		46 (31-59)	46 (27-62)	48 (37-62)	52 (45-62)	49 (41-53)	46 (33-51)	44 (27-56)
BMI, mean (min – max) kg/m^2		26.2 (21.4-37.0)	26.1 (19.0-34.3)	24.2 (19.0-27.7)	27.9 (24.7-30.3)	24.6 (20.8-29.4)	25.5 (19.6-29.4)	26.4 (21.0-34.3)
Smoking habit, N (%)	**non-smoker**	6 (67)	32 (54)	2 (50)	4 (67)	5 (83)	5 (62)	16 (46)
	e-cig smoker	0 (0)	7 (12)	0 (0)	0 (0)	0 (0)	2 (25)	5 (14)
	cigarette smoker	3 (33)	19 (32)	2 (50)	2 (33)	1 (17)	1 (13)	13 (37)
	missing	0	1 (2)	0	0	0	0	1 (3)
Cigarette/day[a], mean (min – max) N		12 (2-20)	13 (3-25)	14 (12-15)	7 (3-10)	8	20	15 (4-25)
Cigarettes smoked before the shift, mean (min – max) N		1 (1-2)	2 (0-10)	2 (1-2)	2 (1-2)	1	3	3 (0-10)
Cigarettes smoked during the shift, mean (min – max) N		4 (1-6)	5 (0-10)	6 (5-7)	4 (2-5)	3	5	6 (0-10)
COT-U, µg/L median (min-max); % > LOQ	**non-smoker**	0.38 (<0.1-1.11); 83	0.35 (<0.1-3.05); 69	1.02 (0.84-1.19); 50	0.20 (<0.1-1.74); 50	0.60 (0.15-1.45); 80	<0.1 (<0.1-1.48); 40	0.27 (<0.1-3.05); 69
	e-cig smoker	-	1530 (1108-2794); 100	-	-	-	2082 (1530-2634); 100	1442 (1108-2794); 100
	cigarette smoker	2023 (1789-2246); 100	1554 (401-4915); 100	2900 (1796-4020); 100	827 (687-968); 100	675 (100)	3619 (100)	1554 (401-4915); 100

REC= exhaust oil receiving, PLANT= remote and on-site plant control, MAN= plant maintenance supervising, LAB= oil quality control, DEL= regenerated oil storage and delivery.
a= only for cigarette smokers

Table 2. Personal exposure to airborne pollutants and concentrations of urinary biomarkers in all subjects and in subjects stratified according to their job and to their smoking habit.

		Administrative workers	Plant workers	Plant Units				
				REC	DEL	LAB	MAN	PLANT
		Personal monitoring						
Number of samples, N (%)		6 (67)	52 (88)	4 (100)	6 (100)	6 (100)	6 (75)	30 (86)
Benzene µg/m^3 median (min-max); % > LOQ		<0.9 (<0.9-0.9); 17	<0.9 (<0.9-6.3); 44	1.3 (<0.9-6.3); 50	<0.9 (<0.9-2.1); 33	<0.9 (<0.9-<0.9); 0	<0.9 (<0.9-1.7); 33	1.0 (<0.9-3.9); 57
		Biological monitoring						
Number of samples, N (%)		9 (100)	58 (98)	4 (100)	6 (100)	6 (100)	8 (100)	34 (97)
BEN-U µg/L median (min-max); % > LOQ	**All**	0.086 (0.034-0.712); 100	0.094 (<0.015-3.095); 89	0.569 (0.111-2.373); 100	0.137 (0.039-0.956); 100	0.043 (0.017-0.094); 100	0.073 (0.051-2.402); 100	0.101 (<0.015-3.095); 97
	Non- smokers	0.073 (0.034-0.209); 100	0.061 (<0.015-0.245); 78	0.122 (0.111-0.132); 100	0.083 (0.039-0.179); 100	0.041 (0.017-0.046); 100	0.081 (0.051-0.101); 100	0.059 (<0.015-0.245); 94
	e-cig smokers	-	0.064 (0.046-0.893); 100	-	-	-	0.062 (0.060-0.064); 100	0.065 (0.046-0.893); 100
	smokers	0.238 (0.108-0.712); 100	0.885 (0.045-3.095);100	1.689 (1.005-2.373); 100	0.824 (0.692-0.956); 100	0.094 (0.094-0.094); 100	2.402 (2.402-2.402); 100	0.875 (0.045-3.095); 100
SPMA µg/g crt median (min-max); % > LOQ	**All**	0.09 (<0.10-3.19); 56	0.15 (<0.10-9.67); 68	3.13 (<0.10-9.67); 100	0.19 (<0.10-1.44); 83	<0.10 (<0.10-1.81); 17	<0.10 (<0.10-6.32); 38	0.17 (<0.10-8.46); 79
	Non- smokers	<0.1 (<0.1-0.13); 33	<0.1 (<0.1-0.70); 46	0.22 (<0.1-0.37); 50	0.15 (<0.1-0.30); 75	<0.1 (<0.1-<0.1);0	<0.1 (<0.1-0.70); 20	<0.1 (<0.1-0.33); 53
	e-cig smokers	-	0.14 (<0.1-0.35);86	-	-	-	0.10 (<0.1-0.14);50	0.16 (0.10-0.35);100
	smokers	1.37 (0.20-3.19);100	1.81 (0.12-9.67);100	7.78 (5.88-9.67);100	0.83 (0.21-1.44);100	1.81 (1.81-1.81);100	6.32 (6.32-6.32);100	0.80 (0.12-8.46);100

REC= exhaust oil receiving, PLANT= remote and on-site plant control, MAN= plant maintenance supervising, LAB= oil quality control, DEL= regenerated oil storage and delivery.

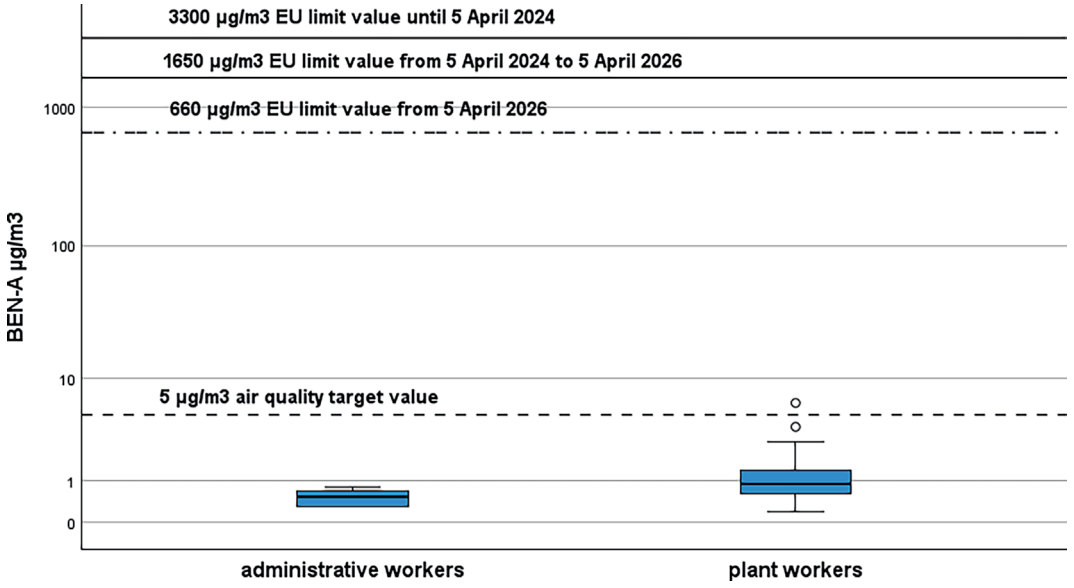

Figure 1. Box-plot of the exposure to airborne benzene in the investigated subjects in comparison with the air quality target value (5 µg/m^3), and the occupational limit values according to EU Directive 2022/431 (3300, 1650, and 660 µg/m^3, corresponding to 1, 0.5, and 0.2 ppm, respectively).

3.3. Biological Monitoring

BEN-U and SPMA levels are reported in Table 2. Urinary samples were available for 67 subjects out of 68 workers. Analytes were above the LOQ for 100 and 89% of samples in administrative and plant workers for BEN-U, and in 56 and 68% of samples in administrative and plant workers for SPMA.

Considering all subjects, BEN-U and SPMA median levels were not different between administrative and plant workers (p=0.731 and p= 0.332 for BEN-U and SPMA, respectively). BEN-U and SPMA values were mostly below the respective biological value equivalent (DFG EKA 7.5 µg/L and ECHA BLV 0.7 µg/L for BEN-U; DFG EKA 45 µg/L and ECHA BLV 2 µg/g crt for SPMA) or biological limit value (ACGIH BEI, 25 µg/g crt for SPMA) (Fig. 2 and 3). In workers, no difference was found among the different job tasks.

When considering the smoking habit, both BEN-U and SPMA levels were higher in plant workers smokers of traditional cigarettes (median 0.885 µg/L and 1.81 µg/g crt, for BEN-U and SPMA, respectively) than in non-smokers (0.061 µg/L and <0.1 µg/g crt) and e-cig smokers (0.064 µg/L and 0.14 µg/g crt) (p<0.001), while no difference between non-smokers and e-cig smokers was observed (p=0.708). In administrative workers too, both BEN-U and SPMA levels were higher in smokers of traditional cigarettes (median 0.238 µg/L and 1.37 µg/g crt, for BEN-U and SPMA, respectively) than in non-smokers (0.073 µg/L and <0.1 µg/g crt) (p=0.043 and 0.076) (Fig. 4 and 5). For both BEN-U and SPMA, values in non-smokers were always below the respective ECHA Biological Guidance Value (BGV) (0.3 µg/L for BEN-U and 0.5 µg/g crt for SPMA) [14].

Considering only non-smokers, BEN-U and SPMA levels were no different between administrative and plant workers (p=0.393 and 0.179, respectively) and among job tasks in plant workers (p=0.150 and 0.336, respectively).

3.4. Pearson's Correlation

In all subjects, significant correlations were found between BEN-U and SPMA (expressed as a function of creatinine) (r=0.748, p<0.001) and between both biomarkers and COT-U (r=0.648, p<0.001 for BEN-U, and r=0.697, p<0.001 for SPMA,

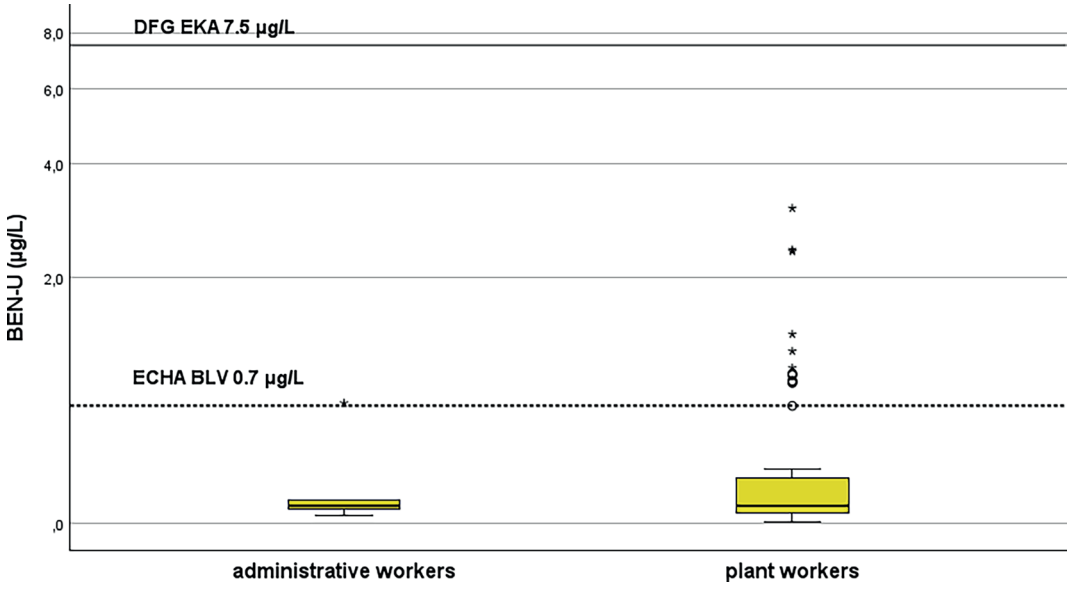

Figure 2. Box-plot of urinary benzene (BEN-U) in the investigated subjects in comparison with the DFG EKA value (7.5 µg/L, corresponding to an exposure to 3300 µg/m^3) and the ECHA Biological Limit Value (0.7 µg/L, corresponding to an occupational exposure limit of 165 µg/m^3 air benzene).

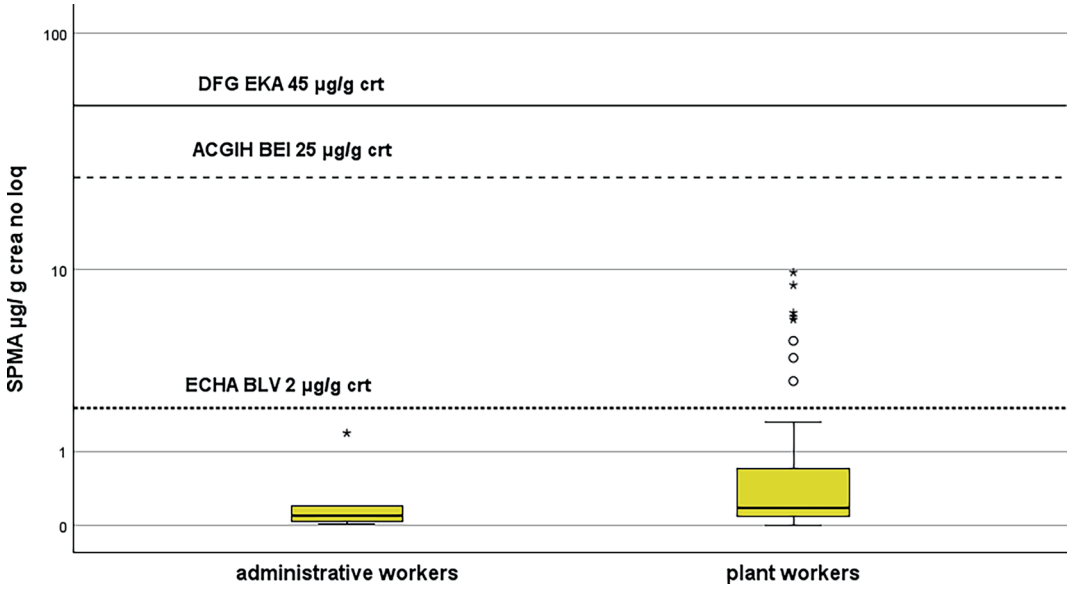

Figure 3. Box-plot of SPMA in the investigated subjects in comparison with the DFG EKA value (45 µg/g crt, corresponding to an exposure to 3300 µg/m^3), the ACGIH BEI (25 µg/g crt, corresponding to an exposure to 1650 µg/m^3) and the ECHA Biological Limit Value (2 µg/g crt, corresponding to an occupational exposure limit of 1650 µg/m^3 air benzene).

respectively). A small but significant correlation was found between BEN-A and BEN-U (r=0.283, p=0.033), and between BEN-A and SPMA (r=0.356, p=0.007).

Considering only non-smokers, the correlation between BEN-U and BEN-A (r=0.128, p=0.493) and between BEN-A and SPMA (r=0.323, p=0.082) was lower than considering all subjects.

4. Discussion

This study assessed the exposure to benzene of workers employed in the re-refining of exhaust oil by measuring both personal exposure and urinary biomarkers. As far as we know, this is the first time workers from this industrial setting have been investigated.

The exposure to airborne benzene was very low and less than 50% of samples had a measurable concentration. In plant workers, both the median and maximum levels found (<0.9 and 6.3 μg/m^3) were more than one thousand times lower than the EU occupational limit value for benzene (3300 μg/m^3) (Fig.1), but also far below the revised limit value (600 μg/m^3) to enter in force in 2026. Moreover, in all subjects but one, median benzene exposure was also lower than the European air quality target value (5 μg/m^3). This result was expected in some way, as worker activities most frequently occurred near closed systems, as it is normally found in refinery plants [23].

However, a higher, even if not significant, proportion of samples had detectable values in plants than in administrative workers, thus showing the possible occurrence of occupational exposure to benzene, although well controlled. Among plant workers, in particular, only workers from the REC and the PLANT unit had quantifiable levels of benzene in at least 50% of samples (Table 2). This could be reconducted to the job tasks of these workers, as REC workers deal directly with the exhaust oil, while PLANT workers may deal with exhaust oil during some specific and short-duration operations, such as the substitution of dirty filters or oil sampling. The exposure to airborne benzene can be considered negligible for LAB workers, for which no samples above the limit of detection were found.

Previous studies on personal exposure to benzene in this setting are not available. However, this peculiar occupational setting can be considered in some way similar to that of the petroleum refinery industry. In this regard, the results of this study are much lower than those reported for workers at four refinery plants in the U.S. (mean value 0.21 ppm or 0.67 mg/m^3) [23], at a Swedish plant (mean values 3.6-74.5 μg/m^3, depending on the job task) [24], at Italian plants (median 18.5-25 μg/m^3) [25, 26], and at offshore oil and gas installations in Norway (geometric mean 12.7 μg/m^3) [27]. Some of these studies also reported a percentage of detectable samples below 50% [23, 24].

Biological monitoring of exposure was performed using two biomarkers, SPMA and BEN-U. Both biomarkers are recognised as sensitive and specific biomarkers of benzene exposure and have been used in both occupational and environmental settings [25, 28]. SPMA is recommended by ACGIH, with 25 μg/g crt as BEI equivalent to the threshold limit value (TLV) as the time-weighted average (TWA) during an 8 h work shift of 0.5 ppm benzene [12]. A value of 45 μg/g crt has been proposed by DFG as a biological value equivalent (EKA) for exposure to 3300 μg/m^3 (1 ppm) benzene [13], while a value of 2 μg/g crt has been suggested by ECHA as a biological limit value (BLV) corresponding to an occupational exposure limit of 0.05 ppm (160 μg/m^3) air benzene [14]. For BEN-U, DFG has proposed a value of 7.5 μg/L as a biological value equivalent (EKA) for an exposure to 3300 μg/m^3 (1 ppm) benzene, ECHA has suggested 0.7 μg/L as a BLV corresponding to an occupational exposure limit of 0.05 ppm air benzene, while ACGIH does not lists BEN-U among the recommended biomarkers of exposure for benzene [12, 13, 14].

Median levels in samples from the investigated subjects were 10- to 100-fold lower than the respective limit values, and levels in plant workers were not different from those of the administrative workers, underlying a low and controlled exposure to benzene, in agreement with the airborne measurements. Moreover, for non-smokers, both plant and administrative workers, values were always below the ECHA Biological Guidance Value for non-smokers [14].

Like personal exposure, biological monitoring has never been performed in this industrial setting. Previous studies in petrochemical plants reported

BEN-U median values in the range 0.15-0.31 µg/L (non-smoker workers) [25, 26], similar to our results, and for SPMA in the range 0.10-8.65 µg/g crt [25, 26, 29, 30].

The low but significant associations found between biomarkers and airborne benzene underline the reliability of both biomarkers, even at the low exposure levels reported here. The relatively low correlation coefficients ($0.356<r<0.283$) are justified, considering that multiple confounders may affect the relationship between air and urinary analytes, especially considering their low levels. We note that the correlation coefficient between BEN-U and BEN-A was similar to what was recently reported for the general population [31].

Moreover, the low biomarker levels and their significant correlations with air levels show that the possible occurrence of dermal exposure to benzene due to contact with dirty materials or to the contamination of workwear has been well controlled. It should be underlined that dermal exposure for these workers was expected to be low as workers may deal directly with exhaust oil or dirty materials only for some specific operations and appropriate personal protective devices (protective overall, gloves, helmet, and safety shoes) were used. This result underlines the benefit deriving from the application of biological monitoring in occupational settings, where differen sources of exposure can occur.

The biochemical verification of the smoking habit through the measurement of COT-U enabled us to quantify the exposure to cigarette smoke. In non-smokers, median and maximum COT-U levels were consistent with no active exposure or with a passive exposure [19]. In cigarette smokers, median (2023 and 1554 µg/L in administrative and plant workers, respectively) and maximum levels (up to 4915 µg/L in plant workers) were indicative of a mean strong or even very strong addiction to nicotine. The same strong addiction was evident in e-cig users, as their median COT-U levels were similar to that of cigarette smokers.

Cigarette smoking had a great impact on biomarker levels, with higher levels of both BEN-U and SPMA in smokers than in non-smokers. For BEN-U, median levels in smokers were almost 10-fold higher than in non-smokers, while for SPMA, median levels in smokers were almost 20-fold higher than in non-smokers (Figures 4 and 5). Moreover, biomarker levels were more frequently detected in smokers than

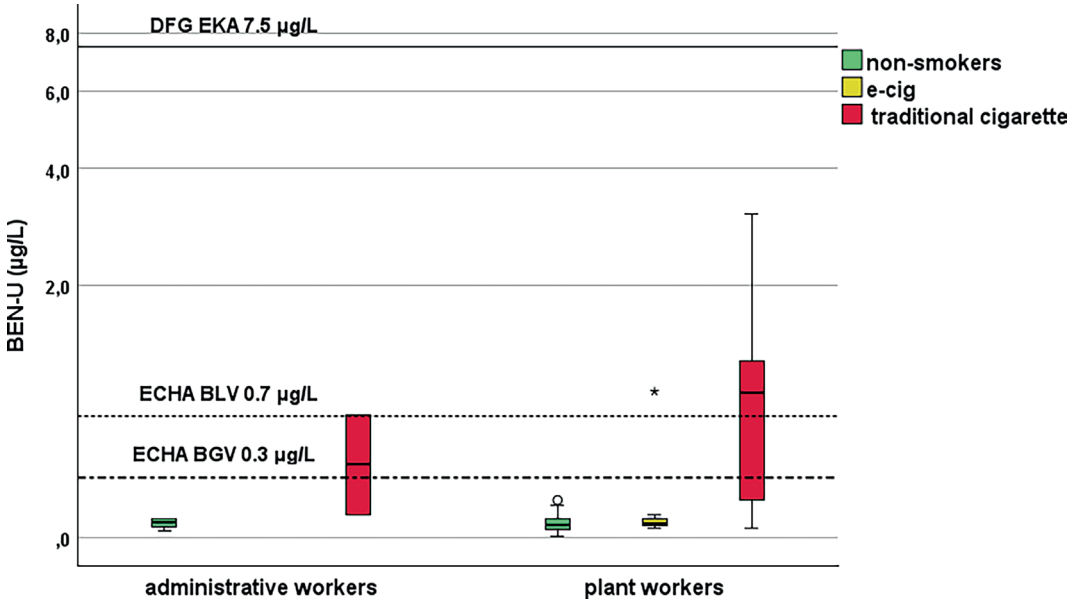

Figure 4. Box-plot of urinary benzene in the investigated subjects stratified according to their smoking habit and in comparison with the DFG EKA value (7.5 µg/L, corresponding to an exposure to 3300 µg/m^3), the ECHA Biological Limit Value (0.7 µg/L, corresponding to an occupational exposure limit of 165 µg/m^3 air benzene), and the ECHA Biological Guidance Value for non-smokers (0.3 µg/L).

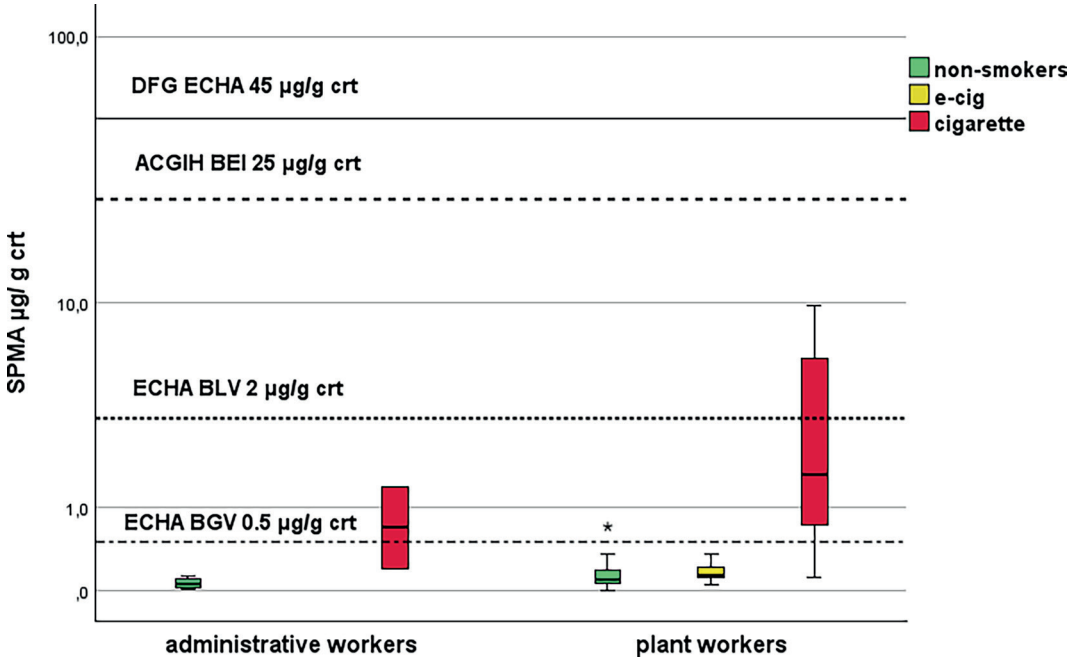

Figure 5. Box-plot of SPMA in the investigated subjects stratified according to their smoking habit and in comparison with the DFG EKA value (45 µg/g crt, corresponding to an exposure to 3300 µg/m^3), the ACGIH BEI (25 µg/g crt, corresponding to an exposure to 1650 µg/m^3), the ECHA Biological Limit Value (2 µg/g crt, corresponding to an occupational exposure limit of 1650 µg/m^3 air benzene), and the ECHA Biological Guidance Value for non-smokers (0.5 µg/g crt).

in non-smokers. These results underline the great influence of cigarette smoking on the internal dose of benzene. The presence of a relatively small group of e-cig smokers among workers allowed us to estimate the impact of e-cig use on benzene exposure. Results showed that levels of BEN-U and SPMA in e-cig users were not different than in non-smokers, so a collateral result of this work is the lack of contribution of e-cig vaping to benzene exposure. This is quite expected, as liquids in e-cigs are vaporised at a temperature lower than that reached by burning tobacco in traditional cigarettes, thus leading to a lower emission of combustion by-products than traditional cigarettes, at least for certain toxics [32, 33]. However, given the great variety of e-liquids possibly used by consumers, the variety of e-cig devices and their uses in terms of battery power settings, and the low numbers of e-cig users in this study, this result should be considered cautiously.

The main strength of this work is the comprehensive evaluation of benzene exposure in workers from this peculiar industrial setting by using a combined approach of personal air monitoring and biological monitoring. For the latter, two up-to-date benzene biomarkers were used, leading to reliable results. On the other side, the main limitation of this study is the relatively small number of subjects investigated. However, the number of workers included in the study coincided with the number of workers employed in the plant, and this allowed us to investigate all the different job tasks performed by workers. It should be mentioned that the low number of subjects is a limitation very common in occupational studies that could be tackled only by performing repetitive studies over time.

5. Conclusion

In conclusion, this study investigated for the first time the exposure to benzene of workers employed in the re-refining of exhaust oil. The results showed that the exposure to benzene was well controlled and

always below the occupational limit values, while the contribution of cigarette smoking to benzene exposure was higher than that of occupational exposure.

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Institutional Review Board Statement: Ethical review and approval were waived for this study due to the conduction of the research in the frame of the risk assessment activity, according to the Italian legislation D.Lgs. 81/08, for the protection of workers' health, under the supervision of the plant occupational health service. The study has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

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