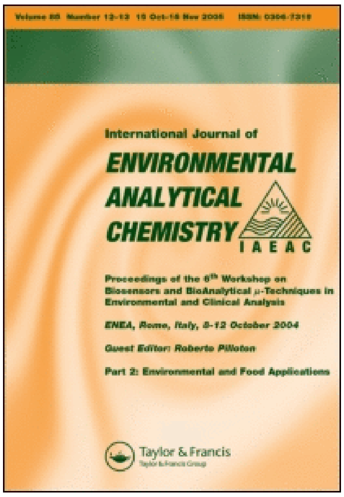

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EXCRETION OF HYDROXY DERIVATIVES OF POLYCYCLIC AROMATIC HYDROCARBONS OF THE MASSES 178, 202, 228 AND 252 IN THE URINE OF COKE AND ROAD WORKERS*

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Urinary PAH-metabolite excretion by non-exposed volunteers, temporarily living on a PAH-poor and PAH-rich diet, respectively, as well as by occupationally PAH-exposed coke plant workers and road workers has been studied. Significant differences in the amount of the metabolites excreted in the urine were detected; the ratio of various metabolites was also found to be different. The mass excretion per liter of the metabolites from phenanthrene was found to be for the unexposed volunteers about 3.5 μg/l, for coke plant workers about 70 μg/l and for road workers about 35 μg/l. For the metabolites of chrysene the values were 0.03 μg/l 2.5 μg/l and 0.09 μg/l, respectively, and for the total metabolites of benzo(a)pyrene: 0.006 μg/l for unexposed persons, 0.37 μg/l for coke plant workers and 0.019 μg/l for road workers.

KEY WORDS: Coke and road workers, urine excretion, hydroxyphenanthrene, hydroxyfluoranthene, hydroxypyrene, hydroxychrysene, hydroxybenzo(a)pyrene.

INTRODUCTION

The goal of this investigation was to establish a parameter for the individual internal occupational burden by polycyclic aromatic hydrocarbons (PAH) in case of cokeries or during road paving, assuming that there is a correlation between the urine excretion of PAH and their metabolites, and the intake from workplace atmospheres.

From a series of papers it is well known that PAH such as phenanthrene, pyrene, chrysene and benzo(a)pyrene are metabolized by rats at various molecular regions resulting in a number of dihydrodiols and, to a lesser extent, of phenols which finally are excreted in the urine, either as such or as their sulfates, as mercapturic acids or glucuronides. Several authors have also studied the urinary excretion of benzo(a)pyrene in man (for a review, see Becher and Bjørseth[1]). Recently, the determination of 1-hydroxypyrene excreted in the urine following exposure to coal tar products has been reported by Jongeneelen,[2,3] while Grimmer *et al.*[4] determined the five isomeric hydroxyphenanthrenes in man and rats.

This paper reports on the excretion of the metabolites of phenanthrene, pyrene,

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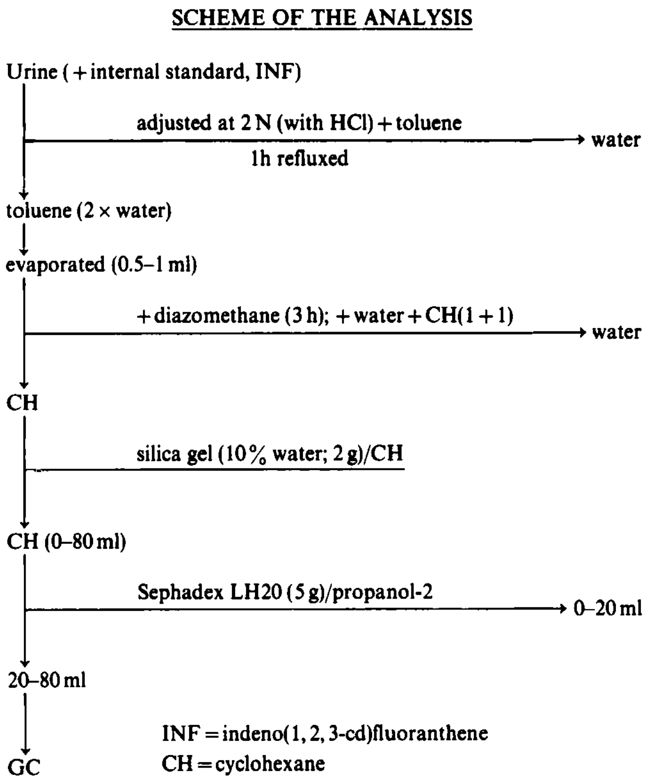

Figure 1 Scheme of the analysis.

chrysene, and of metabolites with mass 282 formed after methylation of phenols deriving from benzopyrenes and/or benzofluoranthenes.

METHODS

In Figure 1 the enrichment procedure for hydroxyderivatives of the PAH is schematically presented. To measure the concentration of metabolites in the urine, conjugates were hydrolysed under 2-N-acidic conditions. Subsequently, hydroxy-PAH were extracted from the urine with toluene and then methylated with diazomethane. The resulting methyl ethers were purified by filtration over silica gel and chromatography on Sephadex LH 20. A gas chromatographic separation of the mixture of five isomeric methoxyphenanthrenes and 1-methoxypyrene is shown in Figure 2.

In the case of hydroxyphenanthrenes, hydroxyfluoranthenes and hydroxypyrene, the concentrations per liter are in the microgram range and therefore the sensitivity of the flame ionization detector is sufficient for their quantification. In the case of the metabolites of higher PAH, such as chrysene and benzo(a)pyrene,

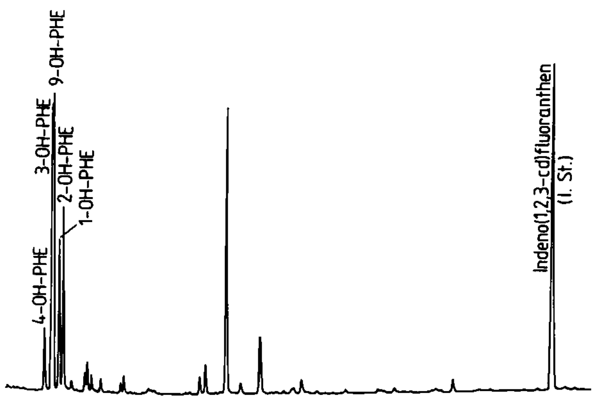

Figure 2 GC separation of isomeric OH-PHE from urine (as methyl ethers), GC: fused silica capillary, SE-54, 25 m × 0.32 mm; film thickness, 0.25 μm; carrier gas, He; injection at 265 °C; detector: FID at 280 °C; oven temp.; 110 °C; temp. programming: 110–160 °C with 10 °C/min., 160–280 °C with 3 °C/min.

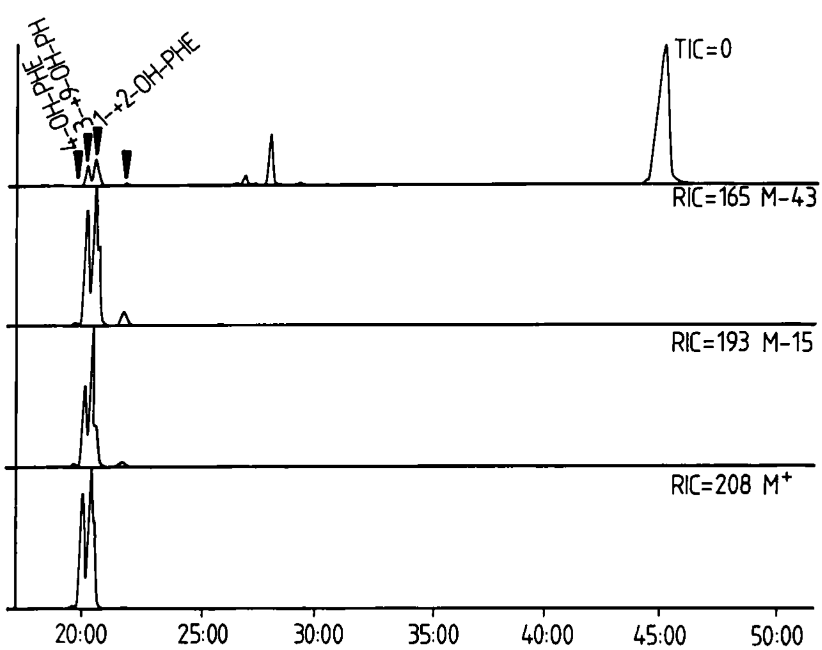

Figure 3 Urine of coke workers after 8 hours exposition. SIM chromatograms of derivatized phenols with mass 208, indicating M^+, (M-15) and (M-43) and the total ion current (upper trace).

the concentrations of the metabolites are in the nanogram and/or picogram range and require mass spectrometric detection using the single ion monitoring mode. In Figures 3 to 6 the identification of further PAH metabolites excreted in urine of coke plant workers is presented.

Figure 3 presents the mass 208 corresponding to the phenanthrene methyl ethers in the lower track. The fragment (M-15) with mass 193 corresponds to an

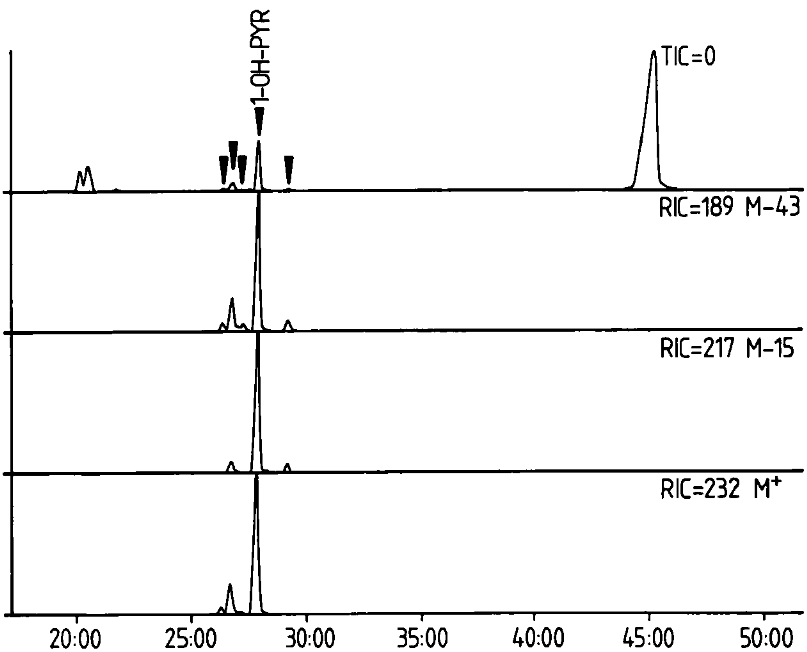

Figure 4 Urine of coke workers after 8 hours exposition. SIM chromatograms of derivatized phenols with mass 232, indicating M^+, (M-15) and (M-43) and the total ion current (upper trace).

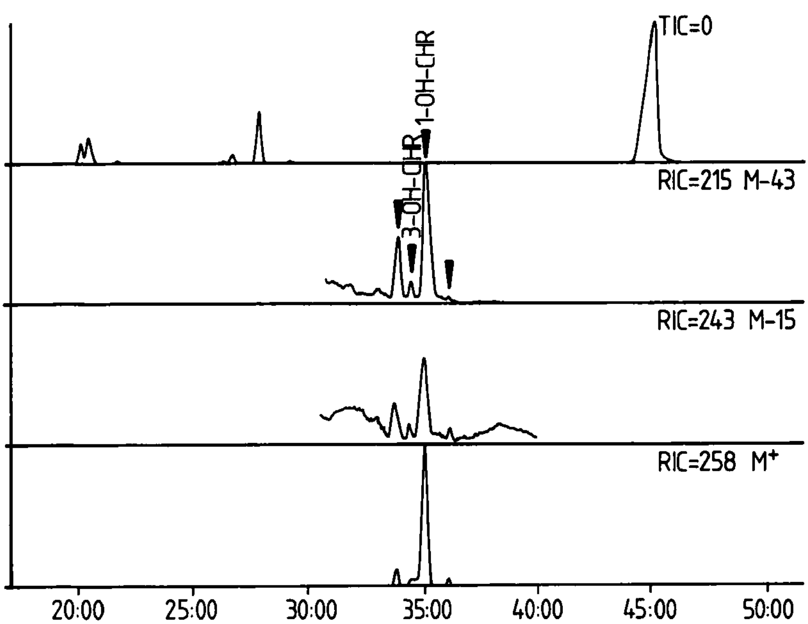

Figure 5 Urine of coke workers after 8 hours exposition. SIM chromatograms of derivatized phenols with mass 258, indicating M^+, (M-15) and (M-43) and the total ion current (upper trace).

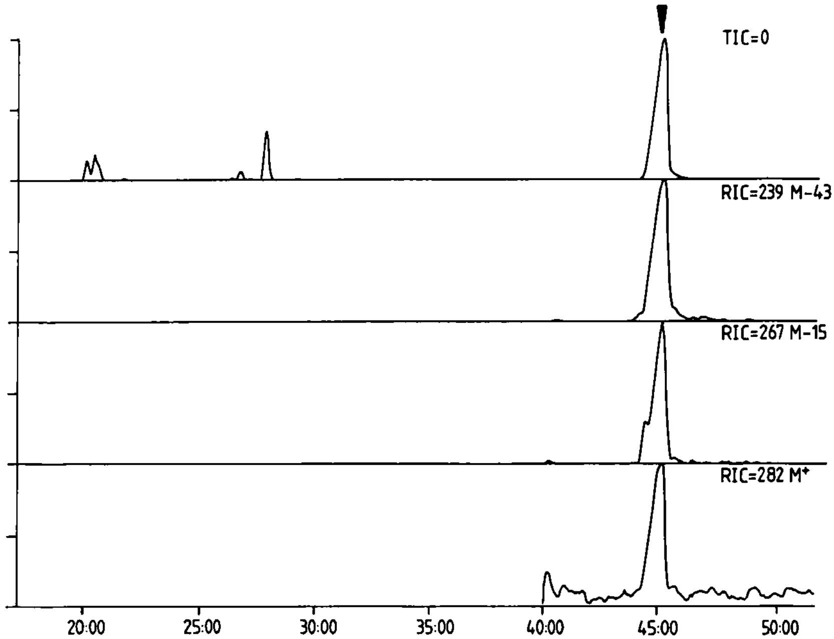

Figure 6 Urine of coke workers after 8 hours exposition. SIM chromatograms of derivatized phenols with mass 282 (indicating M^+, (M-15) and (M-43) and the total ion current (upper trace).

elimination of the methyl group, while the second track shows the (M-43) fragment (elimination of CH_3-C=0). The upper track gives the total ion current.

Figure 4 presents the same type of data for the phenol ethers derived from PAH with mass number 202, e.g. pyrene and fluoranthene, and Figure 5 for phenol ethers derived from PAH with mass 228 which could tentatively be identified as hydroxychrysenes on the basis of their gas chromatographic retention times.

Hydroxy derivatives of PAH with mass 252 have also been detected in the urine of coke plant workers, as is shown in Figure 6. The main peak of this series has been identified by HPLC separation and UV spectrometry as a mixture of 3- and 9-hydroxybenzo(a)pyrene.

RESULTS AND DISCUSSION

To evaluate the influence of dietary PAH on the urinary metabolite excretion, eight volunteers received PAH-poor and PAH-rich diets, and their 24-hour urine was collected. During the first three days they received a PAH-poor diet followed by a three days period of PAH-rich diet as shown in Table 1. The metabolites excreted in the urine of the eight volunteers during the above periods are presented in Figure 7.

The average total amounts of phenanthrene metabolites, daily (24 hours)

Table 1 Polycyclic aromatic hydrocarbons in PAH-poor and PAH-rich diet (μg/kg)

PAH	*PAH-poor diet*	*PAH-rich diet*
Phenanthrene	0.90	8.00
Pyrene	0.32	2.37
Chrysene/triphenylene	0.16	0.98
Benzo(a)pyrene	0.02	0.10

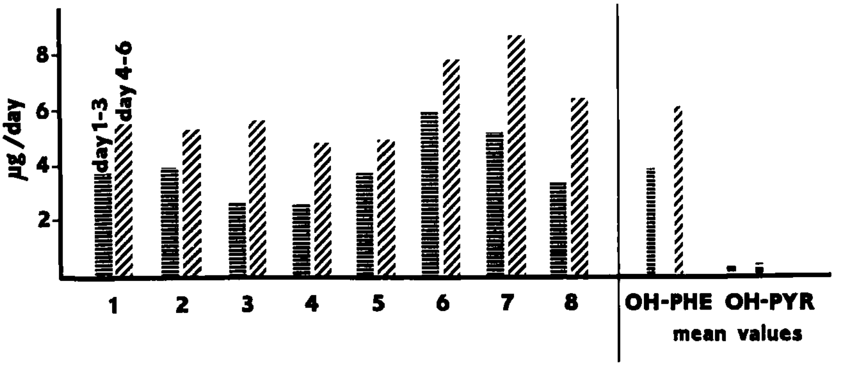

Figure 7 Daily excretion of 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene and 1-hydroxypyrene in urine after PAH-poor (day 1–3) and PAH-rich (day 4–6) diet (μg/day).

excreted in the urine, were 3.84 μg during the first and 6.12 μg during the subsequent period.[5] In other words, a 6-to-9-fold increase of PAH in the diet results only in doubling the amount of metabolites excreted. In contrast to the daily excretion, the excretion per liter is very similar in both groups.

The PAH-rich diet results in higher water intake and, as a consequence, in increasing urine volumes. The figures in column 1 of Table 2 give means of both kinds of volunteers who received either a PAH-poor or a PAH-rich diet during a total period of six days. It is surprising that in the case of the PAH-poor diet a daily intake of about 1 μg phenanthrene/kg resulted in an excretion of about 3 μg of hydroxyphenanthrenes. Probably, most of the excreted hydroxyphenanthrenes resulted from other ways of intake, e.g. inhalation of phenanthrene-containing air.

By contrast, an average amount of 70 μg/l of the five isomeric hydroxyphenanthrenes was found in the daily urine of 25 coke plant workers after an 8-hour exposition—twenty times more than in non-exposed people, as demonstrated in Figure 8.

Eleven out of the 25 coke plant workers were smokers. A mean of 70 μg was found for the total hydroxyphenanthrenes and 7.3 μg/l for 1-hydroxypyrene, respectively. Slightly higher means values were found for smokers and lower for non-smokers.[5] This also holds true for the concentrations of 1-hydroxypyrene which is about one-tenth of that of the total amount of the hydroxyphenanthrenes.

Table 2 Comparison of the urinary PAH-metabolite concentration of unexposed volunteers, coke plant and road workers ($\mu g/l$)

Analyte	*Unexposed volunteers*	*Coke plant workers*	*Road workers*
1-OH-PHE	1.4	35.1	15.2
2-OH-PHE	0.8	15.7	8.7
3- and 9-OH-PHE	1.2	19.2	10.6
4-OH-PHE	0.1	0.5	0.4
OH-PHE (total)	3.5	70.3	34.9
OH-FLU (total)	<0.0001	9.0	1.3
1-OH-PYR	0.3	7.6	2.6
5-OH-CHR	<0.0001	0.8	0.05
6-OH-CHR	<0.0001	0.1	0.003
4-OH-CHR	<0.0001	0.3	0.01
3-OH-CHR	0.01	0.6	0.01
1-OH-CHR	0.02	0.7	0.01
OH-CHR (total)	0.03	2.5	0.09
3- and 9-OH-BaP	0.01	0.3	0.01
6-OH-BaP	<0.0001	0.1	0.01

Unfortunately, it was not possible to collect the urine during 24 hours but only after the working period. Obviously, the internal PAH burden of coke plant workers is many times greater than that of a non-exposed population as indicated by the urinary metabolites.

Similar results were obtained when the urine concentrations of hydroxy metabolites from road workers were studied. A hot blend of bitumen and refined coal tar (140–180 °C) was brought onto the road surface and directly after spraying, stone chips were spread from a truck onto the road. Deposition of the stone chips on the binder was controlled by the fantail operator, while the rakerman controlled the thickness of the chip layer on the road.

In Figure 9 the concentrations of the total hydroxyphenanthrenes and 1-hydroxypyrene in the urine of men after a daily exposition on specific locations are presented. The concentration of the urinary hydroxyphenanthrenes ranges in the same order as observed with the coke workers. However, significant differences between these two groups were found in the amounts of hydroxychrysenes and hydroxybenzo(a)pyrenes excreted. As demonstrated in Figure 10, also shift-dependent metabolite concentrations were detected. Before the beginning of the shift the concentrations of the total hydroxyphenanthrenes was about 50 $\mu g/l$, whereas markedly higher concentrations (90–100 $\mu g/l$) were found at the end of the shift. From animal experiments, in which PAH (phenanthrene, pyrene, chrysene) were administered by various modes,[6,7] it may be concluded that the excretion ends about three days after application.

CONCLUSIONS

Urinary PAH-metabolite excretion by non-exposed volunteers, temporarily living

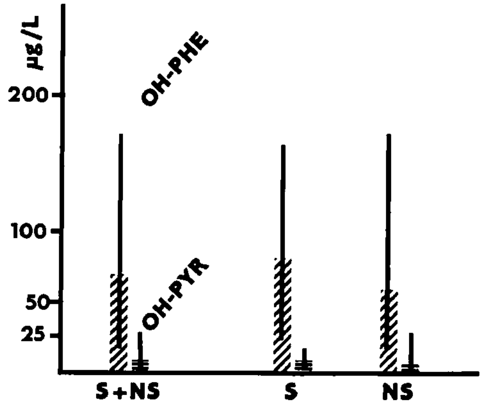

Figure 8 Excretion of hydroxyphenanthrenes and hydroxypyrene in the urine of coke plant workers (smokers: 11, non-smokers: 14) (µg/l).

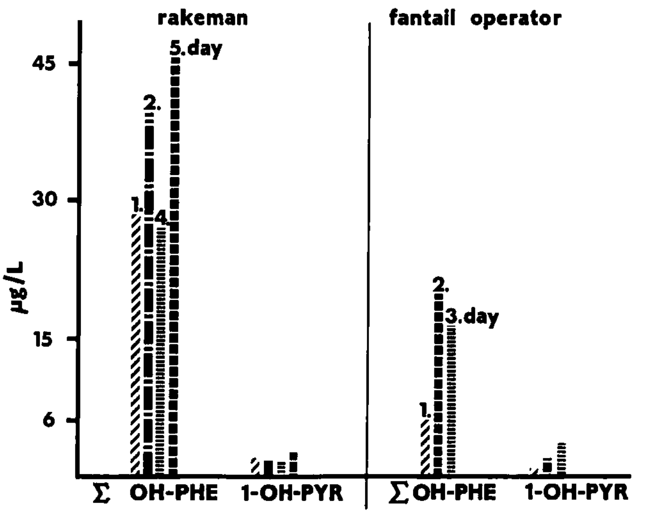

Figure 9 Excretion of hydroxyphenanthrenes and 1-hydroxypyrene in the urine of road workers (rakerman, fantail operator) after the 8 hours shift. Temperature of the blend bitumen and refined coal tar: 140 °C–180 °C.

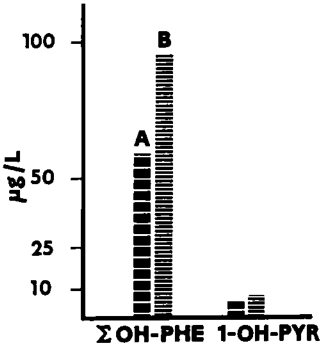

Figure 10 Excretion of hydroxyphenanthrenes and 1-hydroxypyrene in the urine of a fantail operator before (A) and at the end (B) of the shift.

on a PAH-poor and PAH-rich diet, respectively, as well as by occupationally PAH-exposed coke plant workers and road workers has been studied. Significant differences in the amount of the metabolites excreted in the urine, were detected and the ratio of the various metabolites was also found to be different; e.g. in the case of coke workers, the mass excretion of the metabolites from PAH with higher boiling points compared with that of phenanthrene/pyrene was higher than in the case of road workers. This reflects the different concentrations of single PAH present in the working atmosphere. As an example, chrysene and benzo(a)pyrene concentrations are significantly lower in the working atmosphere of road workers. The metabolites recorded for these three groups are presented in Table 2.

The data presented indicate a correlation between the PAH-mass exposition and the mass excretion of PAH metabolites in men. In the case of coke plant and road workers, this method of biological monitoring seems to be suitable for an individual risk assessment at a working place.

Acknowledgements

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