

**EVALUATION OF CERTAIN PARAMETERS OF
EXPOSURE TO MERCURY WITH SPECIAL REFERENCE
TO OCULAR CHANGES**

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ABSTRACT

General clinical and ophthalmological examinations were done of 80 mercury exposed workers (MW) and of 25 control workers (CW). Mercury in blood (Hg-B), mercury in urine (Hg-U) delta-aminolevulinic acid in urine (ALAU) and coproporphyrin in urine (CPU) were also estimated for both groups.

Hg-B and ALAU were not found to correlate highly with mercury exposure. Hg-U was found to correlate with exposure, but due to wide individual variations it can be used only on a group basis. CPU correlated satisfactorily with mercury exposure and its values showed little individual variation among MW suggesting its usefulness both on a group and individual basis for evaluation of mercury exposure.

Ocular changes among MW included mercurialentis (MERC) and bilateral epithelial haze (BEH) in the cornea seen by slit-lamp examination. Both conditions appeared early in the course of mercury exposure although they were not related to other laboratory findings and could be considered as early signs of exposure to mercury.

The time intensity factor (TIF), a weighted index of exposure was found to be a better measure of exposure than either the duration or the intensity of exposure alone.

In recent years overt manifestations of mercurialism such as: salivation, stomatitis, tremor or psychic irritability have become rare in mercury using industries⁸. Moreover, most of these symptoms are non-specific and cannot allow a valid diagnosis of mercurialism on an individual basis. In routine monitoring of mercury exposed workers there is a great need for valid tests to detect as early as possible any deviations from health in their reversible stages. Several tests have been proposed for this. Hg-B and of HG-U have been suggested as indicators of excessive mercury absorption. However, many authors found a low or no correlation between either of the two tests and between the degree of exposure and the clinical signs or symptoms^{8,9,13}. Controversial reports appeared about the activity of erythrocyte delta-aminolevulinic acid dehydratase (ALAD) and the

level of ALAU in the urine of mercury workers^{4,6,14,18,20}. Goldwater and Joselow⁶ found a highly significant correlation between Hg-U and CPU in a group of 52 mercury exposed workers. On the other hand, Stanković and Milić¹⁸ reported that coproporphyrinuria did not exist in mercury exposed workers. Cigula and co-workers⁴, reported a positive correlation between exposure to mercury and total urinary coproporphyrins.

Certain ocular manifestations had also attracted attention as tools for early detection of exposure to atmospheric mercury. Atkinson² reported a coloured reflex (light brownish grey to rosy brown) from the anterior capsule of the lens of mercury workers. The change was found to be bilateral and symmetrical. The finding of a coloured reflex called mercurialentis (MERC) in mercury exposed workers was confirmed by Rosen¹⁶, Lockett and Nazroo¹⁵, Burn³ and Kipling¹². MERC was found not to interfere with vision and it appeared at levels of atmospheric mercury concentrations in which systemic mercurialism does not occur. Hunter¹⁰ suggested that this condition appeared a long time before the onset of general signs of mercurialism. However, the duration of exposure needed for its appearance is a matter of controversy. Lockett and Nazroo¹⁵ found no MERC in workers with less than five years of exposure, whereas Burn³ believes that it appears within a year or two after exposure. Corneal changes were also reported among mercury workers by Rosen¹⁶, Lockett and Nazroo¹⁵ and by Stewart¹⁹. No trials were reported to correlate the ocular changes of mercury workers with other laboratory parameters of mercury exposure.

The conflicting results in previous reports stimulated us to carry out this work with the aim of investigating the validity of the various parameters of mercury exposure and of finding a correlation between them.

SUBJECTS AND METHODS

This study was carried out in a factory engaged in the manufacture of mercury fulminate. Eighty mercury exposed workers (MW) and 25 non-exposed control workers (CW) were the subjects of the study. MW were employed in mixing (N = 12), packing (N = 65) and in the laboratory (N = 3). They were exposed to a concentration of mercury in air of 0.32, 0.07 and 0.04 mg/m³ respectively. Analysis of air in the departments where CW were employed showed no mercury. The mean age of MW was 40.2 ± 8.8 years and that of CW was 36.6 ± 7.6 years. The difference was insignificant (t = 1.969, P > 0.05).

Workers of both groups were subjected to a meticulous clinical examination with a special attention to the mouth, skin and nervous system. Ophthalmological examination followed by slit-lamp biomicroscopy and by ophthalmoscopy under full mydriasis. Blood samples were next taken by venepuncture and each worker provided a sample of urine in a polythene bottle. Hg-B and Hg-U were estimated by the method of dithizone⁵. ALAU and CPU were estimated by the methods of Grabecki and co-workers⁷ and of Stanković and Petrović¹⁷ respectively.

RESULTS

Clinical examination of MW and of CW revealed no relevant findings with the exception of one case of stomatitis and salivation and another case of tremor of both hands among MW. CW showed no ocular abnormalities. Lens changes were the outstanding ocular findings among MW. Rosy brown or pinkish discolouration involved the whole lens or sometimes the finding was limited to the anterior subcapsular disc of the lens. MERC was found in twenty (25%) workers exposed to mercury. In all of them the condition was bilateral. Another ocular finding detected in 14 (17.5%) MW was the presence of bilateral epithelial haze in the cornea. Further ocular findings among MW were non-specific as arcus senilis, post-ulcerative scarring of the cornea of optic atrophy as a result of old retinal affection with glaucoma and chorioretinal disease.

Table 1 shows the results of laboratory findings in CW and MW. The mean values of Hg-B, Hg-U, ALAU and CPU were significantly higher in MW than in CW. In MW there was also a wide degree of scatter of Hg-B, Hg-U and ALAU values as evidenced by the high coefficient of variation of these parameters. The corresponding values for CW were lower. On the other hand, CPU values of MW were less scattered and the coefficient of variation (29.5%) was even lower than that of CW (39.0%).

TABLE 1
Laboratory findings in control (CW) and mercury exposed workers (MW).

Parameter	CW (N = 25)			MW (N = 80)			t
	\bar{X}	S.D.	CV*	\bar{X}	S.D.	CV	
Hg-B (n mol/l)	373.8	164.5	44	558.3	378.8	67.8	2.343**
Hg-U (n mol/l)	215.3	134.6	63	564.3	448.7	80.3	3.672**
ALAU (μ mol/l)	27.4	12.2	44	41.2	25.2	61.1	2.609**
CPU (n mol/l)	68.7	27.0	39	126.1	37.3	29.5	7.072**

*CV = coefficient of variation

**P < 0.01

TABLE 2
Correlation between the duration of exposure of MW and other parameters.

Parameters	r*	t	P
Duration versus Hg-B	0.209	1.887	> 0.05
Duration versus Hg-U	0.267	2.447	< 0.01
Duration versus ALAU	0.185	1.662	> 0.05
Duration versus CPU	0.163	1.459	> 0.05

*r = coefficient of correlation

The effect of the duration of exposure on the various findings is shown in Tables 2 and 3. In Table 2 one can notice that the values of Hg-U correlated positively with the duration of exposure whereas the values of Hg-B, ALAU and

CPU did not correlate similarly. Table 3 shows that cases of MERC increased in percentage with increasing duration of exposure, whereas cases of bilateral epithelial haze (BEH) showed a reverse trend. The mean duration of exposure of workers with either of these two conditions did not differ significantly from other MW without such ocular changes.

TABLE 3
Eye lesions in MW with various duration of exposure.

Duration of exposure (years)	N	Workers with MERC		Workers with BEH	
		N	%	N	%
1-	44	10	22.7	9	20.5
10-	19	5	26.3	3	15.8
20-30	17	5	29.4	2	11.7
Total	80	20	25.0	14	17.5

Table 4 shows the effect of the intensity of exposure on the various parameters studied. Although the mean values of Hg-U and CPU were higher among workers exposed to high mercury in air concentrations, yet the differences were statistically insignificant when F test for analysis of variance was applied. The ALAU values did not differ significantly among workers with different intensities of exposure. The percentage of cases with MERC was nearly similar in the three groups of workers with various intensities of exposure. Cases with BEH occurred only among workers with an exposure of 0.07 mg/m³.

TABLE 4
Laboratory and ocular findings in MW with various intensity of exposure.

Department	Laboratory (N = 3)	Racking (N = 65)	Mixing (N = 12)	F value
Concentration of Hg in air (mg/m ³)	0.04	0.07	0.32	
Mean Hg-U (nmol/l)	533.4 ± 124.6	558.3 ± 388.8	588.2 ± 358.9	0.038*
Mean ALAU (μmol/l)	48.0 ± 23.6	41.2 ± 25.9	39.6 ± 25.9	0.140*
Mean CPU (nmol/l)	123.7 ± 30.1	124.9 ± 35.6	134.1 ± 48.7	0.293*
Workers with MERC (%)	33.3	23.1	33.3	
Workers with BEH (%)	0.0	21.5	0.0	

*P > 0.05

Some MW were found to be exposed to low mercury in air for long periods contrary to others exposed to high concentrations for short periods. Therefore it was found necessary to get a measure of exposure that would take into consideration both the duration and the intensity of exposure. This measure was

calculated for every MW by multiplying the duration of exposure in years of the current intensity of exposure in mg/m^3 in the department in which he was employed. It is called the time intensity factor (TIF). Of course it is an approximate measure of personal exposure liable to certain inaccuracies resulting from shift of some workers between different departments and from the assumption that the current exposure was the same as that prevalent over the whole period of exposure of each worker. As the background concentration of mercury does not represent the actual exposure, it would have been more representative of the actual amount of mercury to which a worker is exposed if personal samplers could be used for 8 hours a day for at least one week in order to be able to compare the figure with the allowed time weighted average (TWA).

TABLE 5
Laboratory and ocular findings in MW with various time intensity factors.

Parameter	Time intensity factor						Test of significance
	0.00-0.99		1.00-1.99		over 2.00		
	\bar{X}	S.D.	\bar{X}	S.D.	\bar{X}	S.D.	
Hg-B (n mol/l)	488.5	334.0	692.9	438.7	682.9	423.7	F = 3.180 P < 0.05
Hg-U (n mol/l)	443.2	355.9	774.2	586.2	822.5	424.2	F = 5.948 P < 0.01
CPU (n mol)	118.2	31.8	138.2	41.4	146.9	46.7	F = 3.795 P < 0.05
Workers with MERC (%)	21.2		26.3		44.4		$Z^2 = 1.182$ P > 0.05
Workers with BEH (%)	13.5		26.3		33.3		$Z^2 = 1.934$ P > 0.05

In Table 5 MW were classified in three groups with increasing TIF. In each group the mean Hg-B, Hg-U and CPU values and the percentage of workers with MERC and BEH were calculated. With the exception of ALAU all parameters showed higher values among workers with higher TIF. This increase was statistically significant for HG-B, Hg-U and for CPU. There was no significant difference between the mean values of ALAU in the three groups. MERC and BEH occurred in a greater percentage among workers with higher TIF. However, because of the small number of ophthalmological findings in each group the differences were found to be statistically insignificant. It should be noted that a marked difference in clinical findings exists between workers with a TIF of less than one and those with a TIF of more than one. On the other hand, the difference between workers with a TIF of one to two and those with a TIF of more than two are small.

Table 6 shows the correlation between various laboratory parameters studied. All parameters were significantly positively correlated with each other. The highest correlation was between Hg-B and Hg-U.

TABLE 6
Correlation between various laboratory findings in MW.

	r*	t	P
Hg-B versus Hg-U	0.933	22.897	< 0.001
Hg-B versus ALAU	0.813	12.329	< 0.001
Hg-B versus CPU	0.719	9.136	< 0.001
Hg-U versus ALAU	0.811	12.243	< 0.001
Hg-U versus CPU	0.723	9.243	< 0.001
ALAU versus CPU	0.824	12.844	< 0.001

*r = correlation coefficient

The laboratory findings in MW with MERC, and in those with BEH were compared with similar findings in other MW and the results are presented in Tables 7 and 8. It is clear from Table 7 that MW with MERC have higher mean values of Hg-B, Hg-U, ALAU and CPU than CW, but these values did not

TABLE 7
Laboratory findings in MW with and without mercurialentis (MERC).

Laboratory	MW with MERC (N = 20)		MW without MERC (N = 60)		t
	\bar{X}	S.D.	\bar{X}	S.D.	
Hg-B (n mol/l)	512.5	249.3	578.3	408.8	0.696*
Hg-U (n mol/l)	523.4	302.5	577.3	494.0	0.451*
ALAU (μ mol/l)	41.9	24.4	40.4	25.9	0.240*
CPU (n mol/l)	129.2	41.4	125.2	82.6	0.216*

*P > 0.05

TABLE 8
Laboratory findings in MW with and without bilateral epithelial haze (BEH).

Laboratory findings	MW with BEH (N = 14)		MW without BEH (N = 66)		t
	\bar{X}	S.D.	\bar{X}	S.D.	
Hg-B (n mol/l)	628.1	353.9	543.4	383.8	0.748*
Hg-U (n mol/l)	685.4	380.4	538.8	465.6	1.086*
ALAU (μ mol/l)	45.8	20.5	39.6	26.7	0.794*
CPU (n mol/l)	152.7	38.8	120.3	34.5	3.158**

*P > 0.05

**P < 0.01

differ significantly from the corresponding values of MW with no MERC. Similar findings can be noted for MW with BEH in Table 8 with the exception of the mean value of CPU which is significantly higher among MW with BEH than among other MW.

Upper normal limits for Hg-B, Hg-U, ALAU and CPU were calculated by using the mean values of these parameters in CW and adding to them the values of two standard deviations. The number and percentage of MW with values of these parameters exceeding the upper normal limits are presented in Table 9.

TABLE 9
Upper normal limits for laboratory findings. The number and per cent of MW with values exceeding these limits.

Laboratory findings	Upper normal limit	MW	
		N	%
Hg-B (n mol/l)	702.9	20	25.0
Hg-U (n mol/l)	484.5	33	41.3
ALAU (μ mol/l)	51.9	22	27.5
CPU (n mol/l)	122.8	35	43.8

It can be noted that in 43.8% of workers CPU values were above the upper normal limit.

DISCUSSION

The finding of a significantly higher mean values of Hg-B and Hg-U among MW is an indication of increased mercury absorption. The levels of Hg-B were not related either to the duration or to the intensity of exposure. This finding is in accordance with the findings of Jacobs and co-workers¹¹, who found no statistically significant correlation between Hg-B and the duration of employment of workers in two factories using mercury. Cigula and co-workers⁴, however, found a strong positive correlation between weighted weekly exposure to air borne mercury and to mercury level in blood of 647 subjects. In the present study the level of Hg-B was significantly higher among MW with higher TIF indicating that weighted exposure is a better measure of mercury exposure than the measurement of air concentration of mercury alone. The levels of Hg-B showed a strong positive correlation with the levels of Hg-U. This confirms the findings of Goldwater and Joselow⁶ who found a similar relation on a group basis only. This finding is however of little importance since both Hg-B and Hg-U are tests of increased absorption. The values of Hg-B exceeded the upper normal limits in only 20 (25%) of MW, whereas a higher percentage of these workers showed abnormalities in other parameters. The coefficient of variation of Hg-B values in MW was higher than that in CW indicating a wider scatter of values. This scatter results from great individual differences in response to mercury exposure. In fact though it is believed that mercury is rapidly cleared

from blood the mechanism of its clearance is not exactly known¹¹. Mercury was found stored in the liver, intestine, kidney, nervous tissue, hair and nails and it may be mobilized from these sites by illness or by large doses of certain drugs⁹. Thus it can be concluded that estimation of Hg-B cannot be regarded as an ideal test for the detection of exposure to mercury.

The levels of Hg-U were found to be positively correlated with the duration of exposure. This finding is in agreement with those of Ladd and co-workers¹³. Although the levels of Hg-U were higher in workers exposed to higher mercury in air concentrations, the differences did not reach the level of statistical significance. In this study the levels of Hg-U were better related to TIF. The mean Hg-U values increased in workers with higher TIF stressing again the importance of weighted exposures. The values of Hg-U in MW showed the highest coefficient of variation among other parameters (80.3%) denoting great individual variation in the urinary excretion of mercury. This finding was reported repeatedly before. Examining 52 MW Armeli and Cavagna¹ found that Hg-U ranged from 0.0 to 623 $\mu\text{g/l}$. Even in five workers with mercurialism they found that Hg-U ranged from 0.0 to 72 $\mu\text{g/l}$. Herrero⁹ found that Hg-U in a group of 109 miners ranged from less than ten to over 500 $\mu\text{g/l}$. He suggested that mercury excretion is not constant and may even continue after termination of exposure. The extreme variation in the levels of Hg-U among MW lowers the validity of this test on individual level as an indicator of the degree of exposure or the existence of chronic intoxication. The fact that a large percentage of MW had Hg-U values above upper normal limit (41.3%) may point to the sensitivity of this test on a group basis. This attitude is adopted by most authors^{8,9}. One may thus conclude that the estimation of Hg-U can be used as a measure of mercury exposure on a group basis only due to marked individual variation in the pattern of urinary excretion of mercury.

The mean ALAU values were significantly higher among MW than among CW. This finding may be related to the findings of Wada and co-workers¹⁰ who reported that ALAD activity is inhibited in cases of long-term mercury exposure. Stanković and Milić¹⁸, however, found no difference in the excretion of ALAU between a group of MW and a group of CW. The same findings were reached by Goldwater and Joselow⁶. In the present study ALAU values did not correlate either with the duration or with the intensity of exposure or with TIF. A lack of correlation of ALAU with exposure to mercury may be related to the findings of Cigula and co-workers⁴ who found no meaningful relationship between ALAD and the weighted exposure to mercury. Lauwerys and Bucket¹⁴ found that ALAU levels were normal in MW with an exposure of 0.05 mg/m^3 mercury in air. In the present study only 27.5% of MW had abnormal ALAU values. It is therefore concluded that estimation of ALAU is not a valid test for detection of early manifestations of mercury exposure.

The mean CPU values were significantly higher among MW than among CW. This finding agrees with those of Goldwater and Joselow⁶ but disagrees with the findings of Stanković and Milić¹⁸ who found only a small difference in CPU between MW and CW. CPU values were not affected either by duration or

by intensity of exposure but they were significantly affected by TIF. This finding is concordant with the findings of Cigula and co-workers⁴ who found a comparatively high positive correlation between total urinary coproporphyrins and weighted exposure to mercury. The values of CPU showed a strong correlation with the values of Hg-B and with those of Hg-U which are parameters of mercury absorption. A similar relation was reported before by Goldwater and Joselow⁶. This finding stresses further the responsibility of mercury in increasing CPU. Abnormal high CPU values were found more often among MW than abnormalities in other parameters indicating a higher sensitivity of this test, taking into consideration its non-specificity. It should also be noted that the values of CPU among MW showed the least coefficient of variation of all laboratory parameters indicating small individual variations. This point may favour the use of CPU as a test of mercury exposure on an individual basis provided that one can rule out other causes of coproporphyrinuria.

In the present study MERC was detected in 25% of MW. This percent agrees with that of Lockett and Nazroo¹⁵, who reported MERC in 24.5% of 49 workers exposed to metallic mercury. Atkinson² recorded an incidence of 52.1% whereas Burn³ discovered the condition in 56 out of 57 men exposed to mercury in a thermometer factory. Of course, variation in figures in different studies may result from differences in the patterns of exposure of studied workers. The percentage of MW with MERC increased with increasing durations of exposure, but the mean duration of exposure of workers with MERC did not significantly differ from that of other MW. Lockett and Nazroo¹⁵ concluded that although the occurrence of MERC is closely related to the duration of exposure, it does not depend on duration alone. They decided that "minimal definite atmospheric concentration of metallic mercury as well as minimal duration of effective exposure are necessary to produce the brown reflex". This fact is quite evident in the present study as it was found that neither the intensity of mercury exposure nor its duration alone were closely related to the development of MERC. When both were combined as TIF the percentage of workers with MERC was markedly higher among workers with increasing TIF. This finding stresses the importance of mercury exposure in inducing MERC. The mean values of various laboratory parameters did not vary between MW with and those without MERC. It seems that the development of MERC is a separate condition not related to changes in biological fluids. This finding confirms the opinion of Lockett and Nazroo¹⁵ that MERC results from direct absorption of mercury via the eyes. Even 22.7% of MW with less than ten years of exposure had MERC. They represent 50% of all MW suffering from this condition. It is therefore quite clear that MERC develops quite early in the course of mercury exposure.

Altogether 17.5% of MW showed BEH in the cornea and 64.2% of them were exposed for less than ten years. The condition was not affected either by duration or by intensity of exposure, but it tended to increase with increasing TIF. With the exception of CPU the mean values of all laboratory parameters were not significantly different between MW with and those without BEH. Corneal changes in mercury exposed workers were reported before by Rosen¹⁶

who described "a questionable yellowish glow" that appeared to be present from the endothelial surface of the wide corneal beam, adjacent to the limbus in a worker exposed to mercury for 37 years. Lockett and Nazroo¹⁵ found that 23% of the mercury workers they examined had marked vascularity at the corneoscleral junction. Stewart¹⁶ reported that chronic exposure to mercury may lead to discolouration of the cornea anterior to the endothelium around the limbus. The significance and pathogenesis of these corneal changes are still obscure. One may therefore conclude that eye changes may occur relatively early among MW and they can be relied upon as indicators of early lead exposure.

In this study it was quite evident that in studying the effects of mercury exposure the use of TIF is a better index than either duration or intensity of exposure alone. A TIF of less than one was found to be the safest exposure level above which all parameters of exposure increased steeply. Applying this level to the current threshold limit values for mercury (0.05 mg/m³) it can be seen that workers can remain only in such atmosphere for 20 years before abnormalities are expected to appear. Lowering the concentration of mercury in air would allow longer exposure of workers.

In conclusion one can say that the findings of the present study show that estimation of Hg-U can be used for evaluating mercury exposure on a group basis only. CPU can also be utilised for this purpose both on a group and on an individual basis. Ocular manifestations also have a diagnostic value in this respect as they appear early in the course of mercury exposure.

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