Anemia Cases after Acute m-Dinitrobenzene Intoxication due to an Occupational Exposure

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Abstract: This is a case report on acute intoxication caused by exposure to m-dinitrobenzene (DNB) that occurred in 1969 in our country. Six workers involved in the intoxication used hammers to remove the DNB crystal layer from the surface of an accidentally overflowed tank and were probably exposed to a large amount of DNB dust. The workers developed cyanosis which was followed by a slight to moderate anemia, viz 1.9–15.5% loss in specific gravity of the whole blood (GB). The magnitude of the anemia was not so different from those reported previously⁴⁾, but prolonged recovery from the anemia was a characteristics of the intoxication cases of the present report. All the workers exposed to the chemical had been followed for ten years and it was confirmed that there was no long term adverse effect being attributable to the exposure.

Keywords: m-Dinitrobenzene—Occupational exposure—Acute intoxication—Anemia—Prolonged recovery from anemia.

MATERIALS AND METHODS

1. The plant and the process of the works:

Intoxication of workers outbroke in a dyestuff plant located in Tokyo. The plant began operations before the Second World War and still in production today with about 500 workers. The exposure occurred in a building where nine employees were working with a black dye called “Deep Black-Ex”. In the same building methamine, an intermediate, was produced from DNB. The production of the chemical is as follows: In a dissolving tank DNB is first dissolved in water by heating. Second, the DNB solution is poured slowly into a reducing tank which is filled with a hydrochloric acid solution and a reducing agent (steel powder). The solution is maintained at high temperature (103–107°C). In this process DNB is reduced to methamine. Finally, the solution is alkalized by adding soda ash and

the methamine solution is separated from the precipitate by a vacuum filter. The capacity of the dissolving tank was approximately 2.5 m³. The tank was covered with a ventilation system. The pressure inside the tank was lower than outside, so that the air flow was from the exterior to the interior through the opening used for supplying materials.

2. The exposure:

About one o'clock in the afternoon on September 10, 1969, while adding DNB to the solution in the tank, the temperature rose unexpectedly and the DNB solution in the tank overflowed. As a result the top of the tank was covered by a DNB crystal layer of 1 to 2 cm in thickness. One worker was splashed with the solution. He suffered first or second degree burn on his back which healed within ten days.

The next day, six workers began cleaning the tank. They used hammers on the surface of the DNB crystal layer to remove it from the tank. The work area was higher than face level and hence the DNB dust would be inhaled. Also, the dust would stick directly to the workers' skin. The workers wore only gauze masks and rubber gloves.

Table 1 shows the dates on which each worker was on duty for the clean up and subsequently exposed to DNB dust. The workers repaired the tank only in the morning. In the afternoon they worked at their ordinary jobs. It took six non-consecutive days to finish the clean up work.

Table 1. Dates of exposure to DNB while repairing the tank.

	Age at Sept. 10 1969	10* WED	11 THU	12 FRI	13 SAT	14** SUN	15** MON	16 TUE	17 WED	18 THU	19 FRI	20*** SAT
K.I.	43		○									
M.M.	50		○	○				○	○	○		
H.Y.	32			○				○				
F.S.	42							○	○			
K.M.	39								○		○	
T.S.	21									○	○	

*: The overflow accident occurred.

**: The plant was closed on Sunday or a national holiday.

***: The process was resumed.

RESULTS

1. Symptoms and clinical findings:

In the evening of September 11, the first day of exposure, one worker (K.I.) became cyanotic at home while drinking an alcoholic beverage just after bath. All the other workers developed cyanosis within one day after their initial exposures, but the work was continued.

On September 19, one day before the clean up work finished, the incident was reported to the health department of the plant. The workers' cloths were washed under the direction of the health department. All workers were given 3 g/day of Vitamin C while cyanosis existed. None received any other specific therapy such as methylene blue.

Beside cyanosis there were few other symptoms. Some complained of a slight headache, nausea and slight dyspnea upon exertion. Cyanosis persisted for about two weeks after the onset in each worker.

About one week after the onset of cyanosis, anemia was apparent among the workers. This was accompanied by palpitation, dizziness, fatigue and languor. These symptoms diminished gradually after the anemia had progressed into the convalescent phase. An iron preparation was administered until the workers recovered.

The health department followed the workers for over a month. Health checks with laboratory tests were done six times from September 19 to October 29. During the period all of the six workers continued their ordinary jobs.

Table 2 shows changes in GB for the exposed workers (including T.K. who suffered from the burn on his back). To estimate a "pre-exposure level" for the each worker, GB values from the closest previous periodic health examination are shown also in the table. Data for June of the same year were available for five of the seven workers and values were available for June of the preceeding year for the other two workers. The standard copper sulfate solution method was used for the GB measurements. The minimum specific gravity of the solution prepared for the examination was 1.048 so that values below the level are expressed as "less than 1.048".

As shown in the table, all six workers who were involved in the clean up of the DNB tank developed anemia 2-3 weeks after the exposure. The magnitude of the anemia ranged from 1.9 to 15.5%. It may be considered that a dose-response relationship is seen between the magnitude of anemia and days involved

Table 2. Changes of GB after the exposure to DNB.

	Pre-exposure* level	1.0xx(x): only xx(x) is indicated						Maximum % loss
		9.19	9.22	9.29	10.8	10.15	10.29	
K.I.	53	52	52	52	52	54	54	1.9
M.M.	53	<48	<48	<48	<48	50	54	>9.4
H.Y.	55	52	50	<48	48	48	54	>12.7
F.S.	56**	54	54	48	48	53	55	14.3
K.M.	58	57	54	49	52	535	565	15.5
T.S.	58	58	56	53	515	53	55	11.2
T.K.	57**	55	58	55	56	57	535	—

*: Examined in June 1969.

** : Examined in May 1968.

Fig. 1. Changes of GB in each exposed worker. Arrows indicate days being on duty for the clean up work.

in the repair work. Worker T.K. who did not participate in the clean up work showed neither cyanosis nor anemia, and worker K.I. who worked on the tank for only one day developed cyanosis but showed less anemia than the others. Daily changes of GB after the exposure are illustrated in Fig. 1 for the six exposed workers. As can be seen, all days of repair work are indicated as arrows. The intervals between the first exposure day and September 19 when the first examination was made are connected by broken lines assuming that the "pre-exposure levels" continued to the first exposure days. The period from the first exposure to the minimum GB measured was 10 to 20 days.

By the last examination date four of the workers did not yet recover to the pre-exposure level. If each gradient in recovery phase is applied after October 29 for an extrapolation, 2 to 14 days more would be necessary to reach the pre-exposure level. From this extrapolation, the period from the first exposure to the recovery may be 30–56 days. The minimum days (30 days) is for worker K.I., who was exposed less than any of the others. If this worker is excluded from the calculation, the average time would be more uniform at around fifty days.

Table 3 shows basophilic stippling of red cells of the workers. Three workers (M.M., H.Y. and F.S.) show increased levels and two (K.I. and K.M.) show also increased level but within normal range. Remaining one (T.S.) shows no

Table 3. Occurrence of basophilic stippling red cells (%) after the exposure to DNB

	Date			
	9.18	9.27	10.8	10.15
K.I.	0.9	0.4	0.8	1.0
M.M.	—	3.4	2.8	2.1
H.Y.	—	—	1.8	2.8
F.S.	—	3.0	2.8	5.3
K.M.	0.4	—	—	1.0
T.S.	0.3	0.3	0.4	0.3
T.K.	0.2	—	0.4	0.2

Table 4. Changes of GOT/GPT (Reitman-Frankel unit) and urobilinogen (in parentheses) after the exposure to DNB.

	Date				
	9.19	9.22	9.29	10.8	10.29
K.I.	45/43(+)	5/ 5(+)	18/18(+)	5/18(+)	(+/-)
M.M.	30/30(+)	18/ 5(+)	18/ 5(+)	18/18(+/-)	(-)
H.Y.	30/18(+)	18/18(+)	18/18(+)	5/5 (+)	(-)
F.S.	18/30(=)	30/18(+)	18/18(+)	5/18(+/-)	(-)
K.M.	18/ 5(+)	18/18(+)	30/18(+)	18/18(+/-)	(+/-)
T.S.	30/18(+)	30/18(+)	18/18(+)	18/18(-)	(-)
T.K.	18/ 5(+)	18/18(+)	18/18 —	18/18(+)	(+)

change. These findings may indicate the increase of basophilic stippling cells.

Results of GOT, GPT and urobilinogen tests are shown in Table 4. GOT and GPT were measured by Reitman-Frankel method. There was no change in the workers from normal limits over the entire period of observation. The results of urobilinogen tests show that all workers, including T.K. who did not participate in the clean up work, were positive from September 19 to September 29, but by October 29 the test results for most workers returned to normal. Bilirubinuria was also observed in almost all the urine specimens when urobilinogen test was strongly positive.

The concentration of plasma protein was evaluated also. The results for all workers were within the normal range (6.5–8.2 g/dl) throughout the period.

2. Follow up:

The plant has continued ordinary periodic health examinations through to the present time. The examination consisted of routine urine and blood tests, blood pressure measurement and an interview by physician. For anyone with positive results from the screening tests, a more detailed examination or examinations would follow. All the workers except worker M.M. who retired in 1977, had been employed continuously for ten years from 1969 to 1979. The attendance rate during the period was 75% to 95%. There was no evidence of delayed effect from the intoxication. The number of GB examinations, mean and standard deviation of GB are shown in Table 5 for each worker. The mean of GB for each worker exceeds the pre-exposure level.

Table 5. The results of periodic health examinations for ten years.

	Attendance to periodic health examinations and rates (%)*	No. of GB examinations	Mean	S.D.
K.I.	19(95)	11	1.0554	0.00112
M.M.	14(88)**	9	1.0550	0.00112
H.Y.	15(75)	6	1.0560	0.00089
F.S.	19(95)	12	1.0561	0.00124
K.M.	17(85)	11	1.0585	0.00082
T.S.	19(95)	5	1.0588	0.00130
T.K.	17(85)	11	1.0568	0.00172

*: Twenty examinations had been carried out for the ten year follow-up period.

**: Retired at 8th year of follow-up. During this period 16 examinations had been carried out.

DISCUSSION

Although no direct evidence of exposure to the chemical in question was available for the cases evaluated in the present paper, the following facts suggest

that an exposure to DNB occurred. First, there was no change in work environment in the building which could have affected the workers' health other than the clean up work following the accidental overflow of the DNB dissolving tank. Second, since the overflow occurred during dissolving DNB into water under high temperature, no other chemical other than DNB was in the solution which crystallized and covered the outside of the tank after the overflow. Third, there was a temporal relationship between the repair work (exposure) and onset of symptoms. Each worker developed cyanosis within one day after the first exposure and the recovery from the anemia which followed the onset of cyanosis happened in the order of the exposure dates. Fourth, after the cessation of the exposure there was no recurrence of the symptoms among the cases and no similar symptoms were observed in any other workers. Fifth, a dose-response relationship was seemed to be present between the number of days exposed and the magnitude of the anemia. Thus, it may be concluded that DNB is probably the sole cause of the acute intoxication.

Inhalation of the dust appears to have played a major part in the route of the absorption. Although the clean up work involved exposure to a large amount of the dust, the workers did not wear an appropriate respirator. Absorption through the digestive tract is also possible through ingestion of large particles trapped in upper respiratory tract. Ishihara¹⁾ reported a high absorption rate of DNB through the skin in a case where a worker dipped her hand into DNB solution. Although there might have been much skin contact with DNB among workers involved in the present report, skin absorption is considered to have played less importance because the contact was not aqueous.

There was no measurement of methemoglobin in the workers included in this report. Some literatures, however, report a relation between the percentage of methemoglobin and clinical symptoms^{2,3)}. That is, 10–15% of methemoglobin is the lowest concentration at which cyanosis will become apparent. At levels from 30–45%, exertional dyspnea may be present. According to the above facts the levels in the workers exposed to DNB in this report was considered to be about 40%.

Anemia was the most significant manifestation of the intoxication in this episode. Wurtz⁴⁾ reported on 187 cases of chemical cyanosis-anemia syndrome caused by various aromatic nitro-, amino-compounds including DNB. In his series, some cases were treated using methylene blue and others were not treated with this drug. He found that methylene blue improves not only methemoglobinemia within a short time, but the drug also shortens the recovery time from the anemia. None of the cases of this report received methylene blue. The extent of anemia in the cases reported here is comparable to that reported by Wurtz, viz a 8.9–19.5% hemoglobin loss. However, prolonged period of recovery from the anemia was the characteristic of the cases in this present series. The average period for recovery from the anemia in the cases of the present paper is supposed to be around 35 days.

It is much longer than the period reported by Wurtz. In his series the average recovery period for the cases not receiving methylene blue was 4.0 days. The agent in the present intoxication was exclusively DNB, a substance thought to be one of the most powerful methemoglobin producing chemicals found in the workplace⁵⁾. Thus, one can infer that recovery from anemia caused by DNB takes a longer period than recovery from anemia caused by other chemicals. Further evidence would be needed to establish this hypothesis as a fact.

Other than the anemia, the only other significant finding from the examinations was bilirubinuria and an increased positive rate of urobilinogen in the urine. Ishihara¹⁾ also reported a temporary increase in Meulengracht's Index in a type of case seen in this report. These findings may suggest that DNB could cause hemolysis and jaundice. The medical literature also suggests that hemolysis and hemolytic anemia can be caused by this chemical²⁾.

Although, hepatotoxicity of DNB has been a matter of controversy^{6,7)}, no evidence of liver damage was observed at least in the GOT and GPT measurements.

In the ten years follow-up after the episode it was revealed that there was no recognizable adverse change in the health status of the workers. However an increase in the level of average GB was observed. Decreased physical labor and improved general work environments due to the introduction of many sophisticated machines may be reasons for the increase of GB.

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