Lethal Poisoning with p-Nitroaniline

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Abstract

A 15 year old boy died after ingestion of an unknown amount of p-nitroaniline with seizures and extremely wide pupils. A MetHb concentration of 36.8 % was photometrically determined in the blood sample collected during autopsy four days after death. By HPLC-DAD and GC-MS p-nitroaniline (4.2 µg/ml) and its metabolites 2-hydroxy-4-nitroaniline (1.5 µg/ml), p-nitroacetanilide (0.38 µg/ml), p-phenylenediamine (0.33 µg/ml) and 4-aminoacetanilide (0.25 µg/ml) were detected in venous blood. A MetHb intoxication was established as the cause of death with the typical secondary symptoms. The origin of the substance and the motivation of the self-administration were not cleared.

1. Introduction

Due to the restrictive regulations for handling of chemicals, lethal acute poisonings with substances from laboratory or industrial practice are seldom in Germany. We report here on a death case after oral p-nitroaniline intake which occurred in Berlin in autumn 2005.

2. Case report.

The 15 year old boy was found in non-responsive state with seizures and loudly crying by his parents at 04.35 h in the morning in his room in the cellar of the house. He had an injury at his head, yellow palms and very wide pupils. Resuscitation attempts were without success and his death was established at 05.30 h in the ambulance on the way to the hospital. A blood sample collected during the resuscitation attempts had a dark brown colour. During inspection of his room, a small bottle with residues of a yellow crystalline substance was found on the window sill (Fig 1a). There was also a small amount of red crystals between the yellow substance (Fig. 1b).

By autopsy, no cause of death could be established. In the brownish gastric content as well as on the surface of the gastric mucosa the same yellow crystals were seen (Fig. 1c).

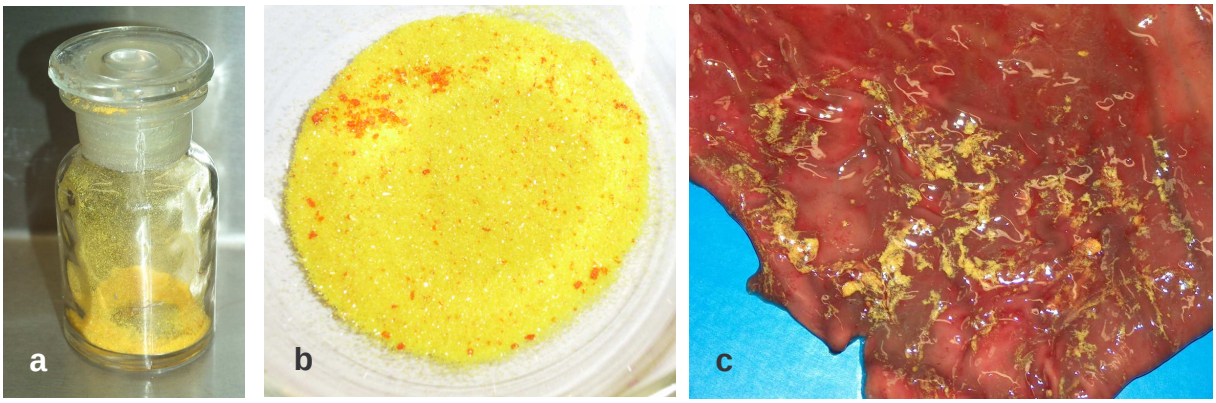

Fig. 1. (a) Flask with yellow substance found on the windowsill of the boys room. (b) Red crystals between the yellow substance. (c) Yellow particles on the gastric mucosa.

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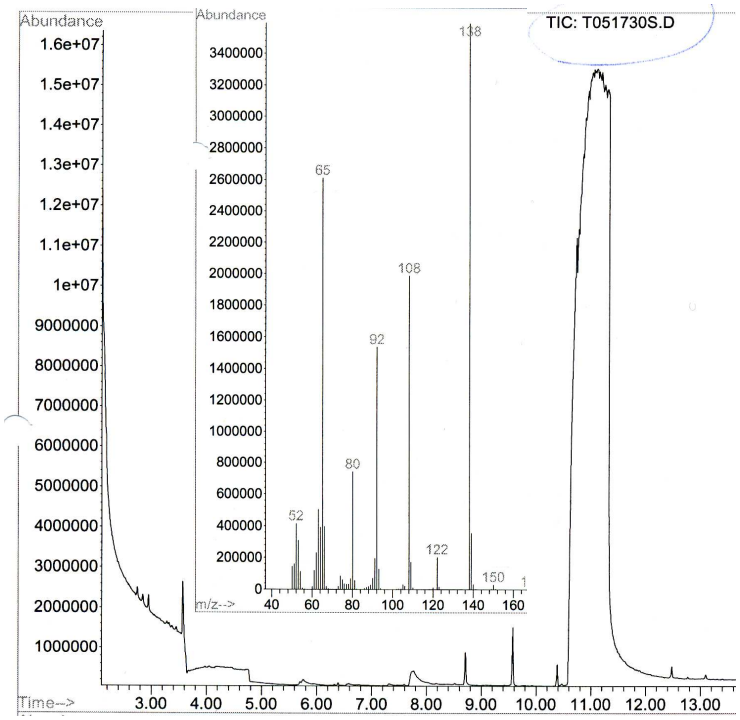
3. Analytical investigations

3.1 Yellow substance and red crystals

The substance was dissolved in ethyl acetate and measured by GC-MS. By library search using the Wiley data base, p-nitroaniline was unambiguously identified (Fig. 2). This was also confirmed by exact agreement of retention time and UV spectrum with the reference substance in the HPLC-DAD chromatogram. The red crystals were identified as potassium dichromate in the same way based on agreement of sample and library UV spectrum in HPLC-DAD.



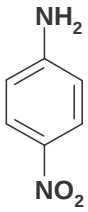


Fig. 2. Identification of the yellow substance by GC-MS as p-nitroaniline (M+ = 138, Hit #1 in Wiley library).

3.2 Systematic toxicological analysis

The body fluids and tissue samples collected during autopsy were systematically investigated for alcohol, illegal and medical drugs and other poisons according to standard procedures. The relevant results are given in Table 1.

Table 1: Results obtained by systematic toxicological analysis

Method	Result
Alcohol (GC and ADH)	Venous blood 0,0 mg/g; urine 0,0 mg/g
EMIT of kidney fluid	Benzodiazepines positive
GC-MS-Analysis for illegal drugs	Negative
Photometric determination of Met-Hb	36,8 %
Systematic analysis by HPLC-DAD	- Diazepam in venous blood 0,04 µg/ml - p-Nitroaniline in venous blood 4,2 µg/ml - Metabolites of p-nitraniline - No indication of further toxic substances
GC-MS-screening of the blood sample	- Basic extract: no toxic substances - Acidic extract: p-nitroaniline and metabolites

Alcohol and the usual illegal drugs were not detected. By photometric determination of Met-Hb 36.8 % were measured one week after death. In a later repetition, a considerable decrease was found. This leads to the conclusion that at the time of death the Met-Hb concentration was probably higher than 36.8 %. Diazepam was obviously administrated during resuscitation for treatment of the seizures. Therefore, it was concluded that the death was caused by the oral intake of p-nitroaniline and its metabolites.

3.3 Analytical determination of p-nitroaniline and its metabolites

A systematic study about the metabolism of p-nitroaniline in human was not found in literature. From animal experiments and in analogy to other aromatic nitro and amino compounds, aromatic hydroxylation, a reduction of the nitro group and acetylation of the amino group are expected (Fig. 3). Despite the short survival time, metabolites should be found to a detectable degree because of the first pass metabolism after oral intake.

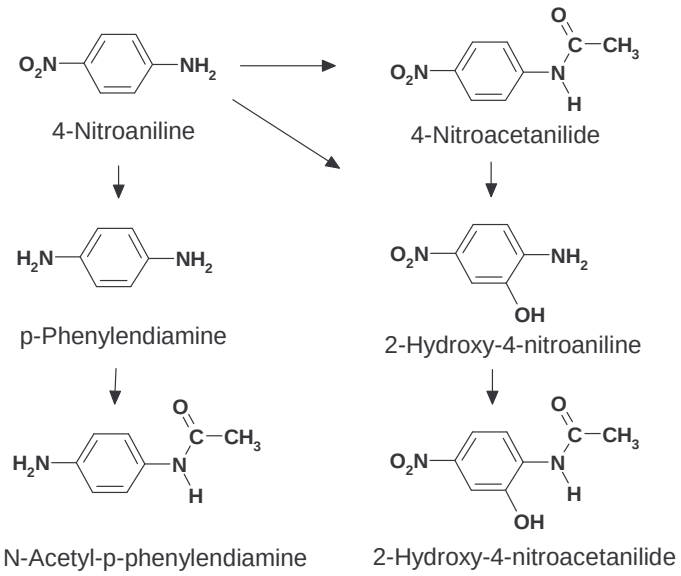


Fig. 3. Expected metabolism of p-nitroaniline

As reference compounds, p-nitroaniline, p-nitroacetanilide, 2-hydroxy-4-nitroaniline, p-phenylenediamine and N-acetyl-p-phenylenediamine were commercially available from Aldrich (Deisenhofen, Germany). The HPLC-DAD analysis was performed according to the conditions described in a previous paper. Besides the parent compound, p-nitroacetanilide (M3) and 2-hydroxy-4-nitroaniline (M4) were identified based on the UV spectra and retention times (Fig. 4).

Because of the low extraction yield and matrix disturbances at the very short retention times, p-phenylenediamine could not be identified by HPLC-DAD. Therefore, this compound was analysed by GC-MS using 2,5-dimethyl-p-phenylenediamine as internal standard.

1 µg internal standard and 0.2 ml tris solution (0.1 M) were added to 1 ml blood and extracted with 1 ml ethyl acetate/chloroform (1:1 v/v). After evaporation of the organic phase, the residue was derivatized with trifluoroacetic acid, again evaporated and the residue dissolved in ethyl acetate for GC-MS measurement. A capillary column HP5-MS was used with the temperature program 2 min at 70 °C, 15 °/min to 300 °C. For the detection in SIM mode the following m/z were used: p-nitroaniline-TFA: 234, 205 and 165; p-phenylenediamine-2TFA: 300, 203, 108; N-acetyl-p-phenylenediamine-TFA: 246, 204 and 107; 2-hydroxy-4-nitroaniline-2TFA: 300, 211, 181; 2,5-dimethyl-p-phenylenediamine-2TFA (int. std.): 328, 259 and 231. A linear calibration curve with a detection limit at about 1 ng/ml was determined for all four substances.

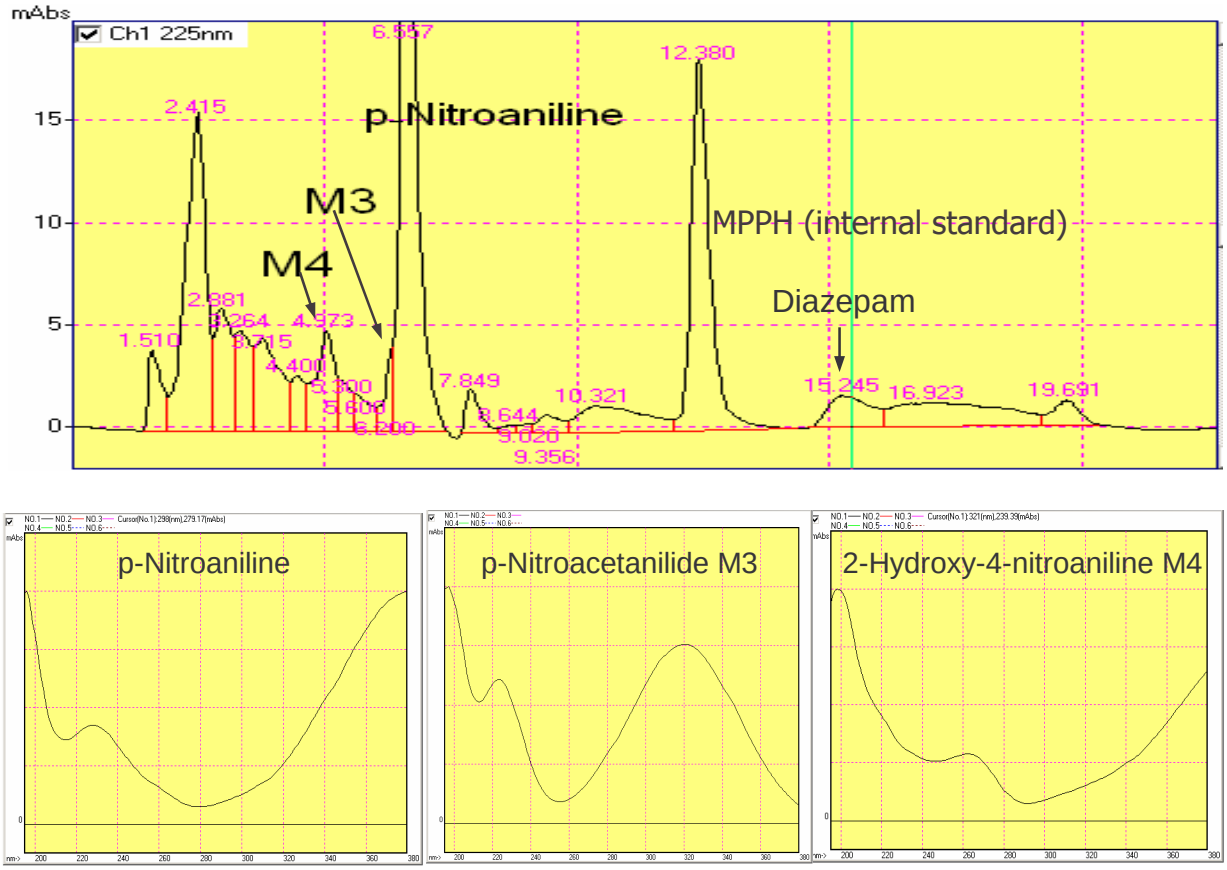


Fig. 4. HPLC-DAD chromatogram at 225 nm of the venous blood sample after extraction with methylene chloride at pH 9 and UV spectra of p-nitroaniline and its metabolites p-nitroacetanilide and 2-hydroxy-4-nitroaniline. The analysis was performed according to the general procedure described in [1].

The concentrations determined by these methods are given in Table 2. In addition to p-nitroaniline, the three metabolites p-phenylenediamine, 4-nitroacetanilide and 4-aminoacetanilide were found in blood in about one order of magnitude lower concentration. However, the concentration of 2-hydroxy-4-nitroaniline is already in the same order as the parent compound, possibly because of the extensive first pass metabolism.

Table 2. Concentrations of p-nitroaniline and its metabolites determined by HPLC-DAD and GC-MS in a death case.

Substance	Venous blood µg/ml	Liver µg/g	Brain µg/g	Kidney µg/g	Gastric content µg/ml
p-Nitroaniline	4.2	0.9	2.4	1.9	613
p-Phenylendiamine	0.33	--	--	--	--
4-Aminoacetanilide	0.25	--	--	--	--
4-Nitroacetanilide	0.38	--	0.2	0.38	0.68
2-Hydroxy-4-nitroaniline	1.5	--	0.4	0.09	3.5

The methods were applied also to liver, brain and kidney tissues and to gastric content. Because of matrix disturbances, the metabolites could not be determined in all samples. Characteristic is the low concentration of p-nitroaniline in the liver as the metabolising organ. The high concentration in gastric content proves the oral intake of the substance.

4. Discussion

Poisonings by p-nitroaniline were described several times in literature [2-6], however, no analytical investigations were performed. The main toxic effect is the induction of the MetHb formation, as it is generally known for aromatic amines and nitro compounds. This was described to be particularly efficient for p-nitroaniline. Furthermore, p-nitroaniline leads to haemolysis. Typical symptoms are cyanosis, shortness of breath, headache, confusion, ataxia, vomiting, dry mouth, cardiac arrhythmia and cardiac arrest. As the cause of death, cardiovascular collapse but not respiratory paralysis is described.

The metabolite p-phenylendiamine should essentially contribute to the toxic effect. According to literature, haemolysis, rhabdomyolysis, acute renal failure, acidosis, oedema of the mucosa in respiratory tract and, due to that, respiratory distress are caused by this substance [7-11]. The MetHb formation seems to be of secundary importance. Pufal and Rochholz measured in a death case after 17 h survival time a p-phenylenediamine concentration of 1,6 µg/ml [11].

In the final report, our case was interpreted in the following way: The death of the 15 year old boy was caused by an oral overdose of p-nitroaniline. Neither the origin of the substance nor the motivation of the intake were cleared. There were no indications of a crime, and a suicide attempt was also improbable. Therefore, an accident in a self-experiment was assumed.

The MetHb formation with the corresponding follow-up symptoms is the predominant toxic effect of the substance. The measured MetHb concentration in blood of 36.8 % is not necessarily lethal. However, it was found in repeated measurements that the concentration in the stored sample decreased. Therefore, the MetHb concentration at the time of death should have been essentially higher. A contribution to the toxic effect by the metabolites formed in the first-pass metabolism, particularly by p-phenylenediamine, has to be taken into account.

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