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# External post mortem artefact: a key issue in hair result interpretation

## *Une contamination post mortem peut influencer l'interprétation des analyses de cheveux*

Pascal Kintz*, Marion Villain, Vincent Cirimele

Laboratoire ChemTox, 3 rue Gruninger, 67400 Illkirch, France

**Abstract – Purpose**: Excluding laboratory mistakes, a false positive hair result can be observed in case of contamination from environmental pollution (external contamination) or after drug incorporation into the hair from the individual body fluids, such as sweat or putrefactive fluid (post mortem artefact). From our 18 years experience of hair testing, it appears that artefact(s) cannot be excluded in some post mortem cases, despite a decontamination procedure. As a consequence, interpretation of the results is a challenge that deserves particular attention. Our strategy will be reviewed in this paper. **Methods**: Three authentic cases are presented to document our hypothesis. **Results**: Case 1: a 24-year old man was found dead in a friend's house. He was not known as a drug addict. The analysis of femoral blood was interpreted as ecstasy poisoning (MDMA = 770 ng/mL, MDA = 56 ng/mL). Segmental hair analysis (GC/MS) was as follows: MDMA = 0.94, 0.87 and 0.90 ng/mg in the 0–3, 3–6 and 6–9 cm, respectively. No MDA was detected. Case 2: at the time of death, cyamemazine, which was never prescribed to the subject, was detected in femoral blood at 3660 ng/mL. The body was exhumed 18 months after burial. Segmental hair cyamemazine analysis (LC/MS-MS) was as follows: 3.1 ng/mg (0–2 cm), 2.9 ng/mg (2–4 cm) and 3.1 ng/mg (4–6 cm). Case 3: the skeleton of a young girl was found in a water well 20 years after her disappearance. 7-amino-flunitrazepam was detected in her cerebral material at 0.67 ng/g. Some hair fibers, attached to the skull were collected. Segmental hair 7-aminoflunitrazepam analysis (LC/MS-MS) was as follows: 15 pg/mg (0–2 cm) and 19 pg/mg (2–4 cm). In all cases, a decontamination procedure with 2 washes of 5 mL of dichloromethane for 5 min was achieved and the last dichloromethane bath was negative for each target drug. From the histories, there was no suspicion of chronic drug use. In all cases, the concentrations detected were homogenous, irrespective of the tested segment. This can be considered as good indicative of potential external contamination. In contrast to smoke, it seems that contamination due to aqueous matrices (sweat, putrefactive fluid, blood) is much more difficult to remove. To explain potential incorporation of 7-aminoflunitrazepam via putrefactive material, the authors incubated negative hair strands in blood spiked at 100 ng/mL and stored at +4 °C, room temperature and +40 °C for 7, 14 and 28 days. After routine decontamination, 7-aminoflunitrazepam tested positive in hair, irrespective of the incubation temperature, as early as after 7 days (233–401 pg/mg). In all periods, maximum concentrations were observed after incubation at room temperature. The highest concentration (742 pg/mg) was observed after 28 days incubation at room temperature. **Conclusion**: It is concluded that a standard decontamination procedure is not able to completely remove external contamination in case of post mortem specimens. Homogenous segmental analyses can be probably indicative of external contamination and therefore a single hair result should not be used to discriminate long-term exposure to a drug. The presence of a metabolite should not be considered as a discrimination tool, as it can also be present in putrefactive material.

**Key words:** Hair, postmortem contamination, decontamination, sedatives, MDMA

**Résumé – Objectif** : Hormis les erreurs d'analyse, un résultat faux positif sur des cheveux peut être observé en cas de contamination par une pollution environnementale (contamination externe), ou après apport de produit dans les cheveux par des fluides corporels, tels que la sueur ou un liquide de putréfaction (interaction post mortem). De nos 18 années d'expérience en matière de tests capillaires, il ressort que de telles interactions ne peuvent pas toujours être exclues malgré les procédures de décontamination. Par conséquent, l'interprétation des résultats est un enjeu qui appelle une attention particulière. Notre stratégie est exposée dans cet article. **Méthodes** : Trois cas concrets sont présentés pour étayer nos hypothèses. **Résultats** : Cas 1 : un homme de 24 ans est trouvé mort au domicile d'un ami. Il n'était pas

* Correspondence: pascal.kintz@wanadoo.fr

connu en tant que toxicomane. L'analyse du sang fémoral a révélé un empoisonnement à l'ecstasy (MDMA = 770 ng/mL, MDA = 56 ng/mL). L'analyse des segments de cheveux par GC-MS a donné les résultats suivants : MDMA = 0,94, 0,87 et 0,90 ng/mg dans les segments 0–3, 3–6 et 6–9 cm, respectivement. Il n'a pas été détecté de MDA. Cas 2 : Au moment du décès, de la cyamémazine, qui n'avait jamais été prescrite au sujet, a été retrouvée à un taux de 3660 ng/mL dans le sang fémoral. Le corps a été exhumé 18 mois après l'inhumation. L'analyse des segments de cheveux par LC/MS-MS a donné pour la cyamémazine les résultats suivants : 3,1 ng/mg (0-2 cm), 2,9 ng/mg (2–4 cm) et 3,1 ng/mg (4–6 cm). Cas 3 : Le squelette d'une jeune fille a été retrouvé au fond d'un puits 20 ans après sa disparition. Du 7-amino-flunitrazepam a été détecté dans la boîte crânienne à un taux de 0,67 ng/g. Des fibres capillaires rattachées au crâne ont été collectées. La recherche d'aminoflunitrazépam dans les segments de cheveux par LC/MS-MS a donné les résultats suivants : 15 pg/mg (0–2 cm) et 19 pg/mg (2–4 cm). Dans tous les cas, une procédure de décontamination par 2 rinçages de 5 min avec du dichlorométhane a été effectuée, le dernier bain de dichlorométhane s'étant révélé négatif aux produits ciblés. Les contextes n'ont pas conduit à suspecter l'usage chronique de produits. Dans tous les cas, les concentrations détectées se sont révélées homogènes dans l'ensemble des segments testés. Ceci peut être considéré comme un bon indicateur de contamination extérieure potentielle. Comparativement à la fumée, il semble que la contamination par des phases liquides (sueur, fluide de putréfaction, sang) soit bien plus difficile à effacer. Pour expliquer l'incorporation de 7-aminofluritrazepam par du fluide de putréfaction, nous avons incubé des mèches de cheveux négatives dans du sang dosé à 100 ng/mL, avec stockage à une température ambiante de +4 °C, et à +40 °C pendant 7, 14 et 28 jours. Après une décontamination standard, du 7-aminofluritrazepam a été détecté dans les cheveux, quelle que soit la température d'incubation, et dès 7 jours (33–401 pg/mg). Sur toutes les périodes, les concentrations maximum ont été observées après incubation à température ambiante. La plus haute concentration (742 pg/mg) a été observée après une incubation de 28 jours à température ambiante. **Conclusion** : Une procédure de décontamination standard ne permet pas d'effacer totalement une contamination extérieure dans un contexte post mortem. Des analyses de segments homogènes peuvent retranscrire une contamination extérieure, et donc, un test capillaire unique ne permet pas de conclure à une exposition de longue durée à un produit. La détection d'un métabolite, qui peut aussi être présent dans les fluides de putréfaction, ne permet pas non plus de conclure dans ce sens.

**Mots clés :** Cheveux, contamination *post mortem*, décontamination, sédatifs, MDMA

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## 1 Introduction

The major advantage of hair testing compared to urine or blood testing for drugs is that it has a larger surveillance window (weeks to months, depending on the length of the hair shaft, against 2–4 days for most drugs in blood and urine). For practical purposes, the two tests complement each other. Urinalysis and blood analysis provide short-term information of an individual's drug use, whereas long-term histories are accessible through hair analysis.

By providing information on exposure to drugs over time, hair analysis may be useful in verifying self-reported histories of drug use in any situation in which a history of past rather than recent drug use is desired. In addition, hair analysis may be especially useful when a history of drug use is difficult or impossible to obtain.

Numerous post mortem applications have been described in the scientific literature where hair analysis was used to document the case: suspicious death, evidence of drug administration, evidence of long-term poisoning, discrimination between single and chronic exposure, demonstration of tolerance, pattern of drug use, crime under the influence of drug [1].

Although there is reasonable agreement that the qualitative results from hair analysis are valid, the interpretation of the results is still under debate owing to unresolved questions such as the influence of external contamination. More research is required before all of the scientific questions associated with hair drug testing will be satisfied. There is still a lack of consensus among the active investigators on how to exclude external contamination.

Contamination of hair would be a problem if from a negative specimen the findings of a drug and/or metabolites(s) will lead to a positive interpretation. It is unlikely that anyone would intentionally or accidentally apply anything to his or her hair that would contain a drug. The most crucial issue facing hair analysis is the avoidance of technical and evidentiary false-positives. Technical false-positives are caused by errors in the collection, processing and analysis of specimens, while evidentiary false-positives are caused by passive exposure to the drug. Approaches for preventing evidentiary false-positives due to external contamination of the hair specimens have been described since 1992 [2]. These criteria do not endorse a general acceptance [3, 4]. Excluding laboratory mistakes, a false positive post mortem hair result can be observed in case of contamination from environmental pollution (external contamination) or after drug incorporation into the hair from the individual body fluids, such as sweat or putrefactive fluid (post mortem artefact). An artefact is a drug present in hair during analysis that do not correspond to the genuine drug present in the body at the time of death.

Most laboratories use a wash step; however, there is no consensus or uniformity in the washing procedures. Among the agents used in washing are detergents such as shampoo, surgical scrubbing solutions, surfactants such as 0.1% sodium dodecylsulfate, phosphate buffer, or organic solvents such as acetone, diethyl ether, methanol, ethanol, dichloromethane, hexane or pentane of various volumes for various contact

times. From the papers in the scientific literature, a single washing step is used; although a second identical wash is sometimes performed. If external contamination is found by analysing the wash solution, the washout kinetics of repeated washing can demonstrate that contamination is rapidly removed. Baumgartner and Hill [2], published that the concentration of drug in the hair after washing should exceed the concentration in the last wash by at least ten times. This was recently confirmed by Tsanaclis and Wicks [5]. It has also been proposed that hair should be washed three times with phosphate prior to analysis to remove any possible external contamination and that the total concentration of any drug present in the three phosphate washes should be greater than 3.9 times the concentration in the last wash [2]. Obviously, washing removes drug from the interior as well as from the exterior surface of hair during decontamination procedure [2].

According to Romano *et al.* [6], even using the most sophisticated decontamination procedures, it is not possible to distinguish a drug-contaminated subject from an active user. However, these results were challenged by Cairns *et al.* [7]. Thus, while a negative result excludes both chronic use and contact with drugs, a positive result cannot be interpreted as a sure sign of drug addiction.

Detection of drug metabolite(s) in hair, whose presence could not be explained by hydrolysis or environmental exposure, were proposed to unequivocally establish that internal drug exposure had occurred. Cocaethylene and nor-cocaine would appear to meet these criteria, as these metabolites are formed when cocaine is metabolised [8]. Because these metabolites are seldom found in illicit cocaine samples, they would not be present in hair as a result of environmental contamination, and thus their presence in hair may be considered as a marker of cocaine exposure. This procedure can be extended to other drugs, such as THC-COOH for cannabis [9]. However, until now, specific metabolites for numerous drugs (opiates, amphetamines, GHB, benzodiazepines...) have not been identified in hair.

As it can be observed from the scientific literature [2–8], several approaches have been proposed to get round the contamination scenario. All are dealing with living subjects and none have been developed for post mortem specimens, collected under very bad conditions. These examples and references apply to environmental contamination and not to incorporation via contact with body fluids.

The authors present here 3 post mortem cases where artefacts could not be excluded and the corresponding interpretative issues, which represent a first proposal to avoid false conclusions.

## 2 Case reports

### 2.1 Case 1

A 24-year old man was found dead in a friend's house. He was not known as a drug addict. The analysis of femoral blood was interpreted as ecstasy poisoning (MDMA = 770 ng/mL, MDA = 56 ng/mL). Hair (9 cm, brown) was collected at the time of the autopsy. Decontamination for about 200 mg of hair involved shaking the specimen in 2 times 5 mL of dichloromethane for 5 min at room temperature. The specimen was dried with absorbent paper following the 1st washing step. Analyses were achieved using GC/MS after sodium hydroxyde incubation in presence of deuterated internal standards, extraction with ethyl acetate and derivatization with HFBA [10]. Segmental hair analysis was as follows: MDMA = 0.94, 0.87 and 0.90 ng/mg in the 0–3, 3–6 and 6–9 cm, respectively. No MDA was detected (LOQ at 0.05 ng/mg). The authors concluded that the presence of MDMA in hair could be explained by excessive sweating associated to hyperthermia (as documented by the interview of his partner) during the time between ingestion and death.

### 2.2 Case 2

At the time of death, during body examination, cyamemazine, which was never prescribed to the subject, was detected in femoral blood at 3660 ng/mL. The body was exhumed 18 months after burial, to verify the absence of wound (request of the judge). During the autopsy, hair (6 cm, black) was collected. Putrefaction was massive and the hair was in contact with organic liquid. Decontamination involved 2 times 5 mL of dichloromethane, as indicated in case 1. Analyses were achieved using LC/MS-MS after phosphate buffer (pH 8.4) incubation, and extraction with dichloromethane/ethyl ether, according to our procedure for neuroleptics [11]. Segmental hair cyamemazine analysis was as follows: 3.1 ng/mg (0–2 cm), 2.9 ng/mg (2–4 cm) and 3.1 ng/mg (4–6 cm). Finger nail (thumb, 55 mg, 3 mm large, obtained after clippings), also collected after exhumation, was negative (LOQ at 10 pg/mg), that is indicative that the subject might be naïve. In addition, there was no medical record indicating cyamemazine prescription. The origin of cyamemazine in hair is likely to result from contact with the putrefactive organic material in a case of acute overdose for suicidal purposes.

### 2.3 Case 3

The skeleton of a 17-year old girl was found in a water well about 20 years after her disappearance. Some cerebral material remained in the skull where 7-amino-flunitrazepam was detected at 0.67 ng/g. Some hair fibers (4 cm, dark), attached to the skull were collected. Tedious investigations from the Police revealed that there was no medical prescription for Rohypnol to the girl. To document the case and to discriminate between therapeutic use and single exposure (chemical weapon to sedate the victim) by a serial rapist, the remaining hair was tested. Decontamination involved 2 times 5 mL of dichloromethane as indicated in case 1. Analyses were achieved using LC/MS-MS after phosphate buffer (pH 8.4) incubation in presence of deuterated diazepam, and extraction with dichloromethane/ethyl ether [12]. Segmental hair 7-aminoflunitrazepam analysis (LC/MS-MS) was as follows: 15 pg/mg (0–2 cm) and 19 pg/mg (2–4 cm). Due to possible

post mortem contamination, all interpretations were found inconclusive, although the concentrations were indicative of occasional exposure.

## 3 Experimental study

To verify if there is any possibility that long-term contact between hair and organic material containing a drug can produce a false positive result, the authors implemented the following protocol. Ten strands of hair (5 cm, brown) were collected from a naïve subject. The cut end was sealed by wax. Three strands were incubated in 10 mL whole blood, spiked at 100 ng/mL with 7-aminoflunitrazepam at 4, 23 (room temperature) and 40 °C for 7, 14 and 28 days. Decontamination involved 2 times 5 mL of dichloromethane. Analyses were achieved using LC/MS-MS after phosphate buffer (pH 8.4) incubation in presence of deuterated diazepam, and extraction with dichloromethane/ethyl ether [12].

## 4 Results and discussion

The routine decontamination procedure of the laboratory involves 2 consecutive washes with 5 mL of dichloromethane for 5 min at room temperature, when about 200 mg of hair are processed. The procedure has been used for 18 years and is efficient for various compounds, including drugs of abuse, pharmaceuticals and doping agents, even it was challenged by Romano *et al.* [6]. In case of dirty specimens (blood stains, vomit stains, ground, etc), the specimens are pre-washed with warm water until a clear effluent is obtained, in addition to the $2 \times 5$ mL dichloromethane washes. In case of suspicion of external contamination, the second dichloromethane wash is analyzed and the ratio total concentration in hair (in ng) to concentration in wash is established. When this ratio is higher than 10, this indicates drug use as opposed to external contamination.

In the three cases and the controlled study, the last dichloromethane wash was negative for the target drug. As hair damage or degradation promotes both contamination and incorporation via drug containing body fluids, a visual evaluation of the specimens was achieved, that did not reveal actual degradation.

The hair of the naïve subject was free of 7-aminoflunitrazepam, with a LOQ at 1 pg/mg. Damage or degradation of the hair during the incubation period was not noticed. The spiked hair tested positive for 7-aminoflunitrazepam, irrespective of the period of incubation and the temperature of incubation. In each of the 9 analyses, the concentration of total 7-aminoflunitrazepam in the last dichloromethane wash was lower than 10 pg. Concentrations are given in Table I and ranged from 233 to 742 pg/mg. In case of single exposure to 1 mg of flunitrazepam, that is enough to seriously impair a victim of drug-facilitated rape, the concentration of 7-aminoflunitrazepam is about 1 to 5 pg/mg [13]. Obviously, contamination can play a major role in interpretation of the findings.

From this experiment, the authors concluded in case 3 that the positive findings of 7-aminoflunitrazepam in the 2 segments is compatible with artefact contamination during the post mortem decomposition of the corpse. The judge in charge of the investigations accepted that the levels in hair could have been derived purely from contacts with body fluids and tissues of the victim and charged the suspected perpetrator of poisoning before killing.

**Table I.** 7-aminoflunitrazepam concentrations (pg/mg) in hair after incubation in spiked blood.

| Time | Incubation at 4 °C | Incubation at 23 °C | Incubation at 40 °C |
|---|---|---|---|
| T0 | < LOQ | < LOQ | < LOQ |
| T + 1 week | 233 | 401 | 308 |
| T + 2 weeks | 404 | 609 | 283 |
| T + 4 weeks | 588 | 742 | 276 |

From these cases and the experimental study, it was concluded that our standard decontamination procedure is not able to completely remove external contamination in case of post mortem specimens nor differentiate without any doubt between artefact(s) or drug use. It is our opinion that the presence of water-containing tissue (blood) may be in favour of contamination when hair is in contact with. The presence of homogenous consecutive concentrations after segmental analysis may be considered as indicative of potential contamination from an individual's body fluids or tissues.

The differentiation between drug use and external contamination has been frequently referred to as one of the limitations of drug testing in hair. The detection of relevant metabolite(s) has been proposed to minimise the possibility of external contamination causing a misinterpretation. Difficulty arises when a metabolite is not detected either due to the absence of specific metabolite or to low doses of the drug used. Moreover, in post mortem toxicology, the presence of a metabolite cannot be considered as a discrimination tool, as it can also be present in the biological (putrefactive) tissues.

From the contamination scenario, it is also possible that post mortem hair gets contaminated from a biological fluid that does not actually belong to the decedent.

## 5 Conclusion

In post mortem toxicology, a single hair result should not be used to discriminate long-term exposure to a drug. It must be emphasized that with a single hair result, it is not possible to determine the exact amount of drug that was used during the previous period. In post mortem toxicology, one should encourage active investigators to perform multi-sectional analyses, which homogenous results can be indicative of contamination. Finally, in some cases, hair analysis from post mortem cases is equivocal, and results should be interpreted with caution. It should also be mentioned that the proposed criterion can be helpful in cases similar to those reported but it may fail or should not be applied when the circumstances surrounding death are fragmentarily known or when there is no evidence at all.

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