Introduction

The toxicological analysis of medicines and chemicals in formaldehyde-treated specimens are a component of forensic toxicology. This review involves two parts: (i) reactions of formaldehyde with substances in tissues fixed with formalin (aqueous formaldehyde solution) and (ii) the stability of substances in formalin-treated tissues and formalin solutions. Because it was well known that formaldehyde reacts with amines to form Schiff bases, it was additionally suggested in 1998 that formaldehyde reacts with primary and secondary amines together with formic acid to form N-methyl substances via the Eschweiler–Clarke conversion, which proceeds at room temperature. The reaction increases with pH and the concentration of formaldehyde. In addition, this report includes both experimental studies and practical cases involving embalmed bodies.

Reactivity of formaldehyde with substances including amines

**Eschweiler–Clarke conversion**

Formaldehyde, together with formic acid, reacts with primary and secondary amines, in a reaction known as the ‘Eschweiler–Clarke conversion’ (7–9), as shown in Figure 1. Primary amines react with formaldehyde as follows (9) (Figure 1, Step 1):

\[
R–\text{CH}_2–\text{NH}_2 + \text{H}–\text{CO}–\text{H} + \text{H}_2\text{O} \\
\rightarrow R–\text{CH}_2–\text{NH}_2 + \text{H}_2\text{C}^+–\text{OH} + \text{HO}^– \\
\rightarrow R–\text{CH}_2–\text{N}^+\text{H}_2–\text{H}_2\text{C}–\text{OH} + \text{OH} \\
\rightarrow R–\text{CH}_2–\text{NH}–\text{CH}_2–\text{OH} + \text{HOH} \\
\rightarrow R–\text{CH}_2–\text{N}^+\text{H} = \text{CH}_2 + \text{HOH} + \text{OH} \\
\rightarrow R–\text{CH}_2–\text{N} = \text{CH}_2 + \text{HOH} + \text{H}_2\text{O}
\]

The imine reaction product then reacts with formic acid (Step 2). This reaction favors alkali conditions (e.g., pH > 5), but it can occur under acidic conditions (pH < 4). The release of CO₂ from formate ions is the key step in the methylation of primary amines (Step 3). Secondary amines react with formaldehyde (Step 4) and form a quaternary ammonium salt [R–CH₂–N(CH₃)₂]⁺. Therefore, the Eschweiler–Clarke conversion does not cause tertiary amines to further react with formaldehyde. Steps 5 and 6 are almost the same as Steps 2 and 3, respectively, as shown in Figure 1.

The conversion has been recognized since 1933 (7–9), but it was not until 1998 that it was found to apply to FFT and FS (10). Takayasu et al. (10) suggested that amitriptyline and imipramine are produced from nortriptyline (Ntp) and desipramine, respectively, in FS at room temperature (RT) by the Eschweiler–Clarke conversion. Conversions of secondary to tertiary amines by the Eschweiler–Clarke reaction were, thereafter, revealed by several researchers using fenfluramine (FF) (11), methamphetamine (MA) (12, 13), sertraline (Srt) (14), fluoxetine (Flx) (15) and 3,4-methylenedioxymethamphetamine (MDMA) (16). However, these papers did not give the particulars of the reaction, for example, the optimum ratio of formic acid to formaldehyde, pH and temperature.

**Other reactions of formaldehyde with primary and secondary amines**

In previous studies, reactions of formaldehyde with primary and secondary amines were presumed to form Schiff bases as follows: addition of primary amines to an aldehyde gives

\[
\text{R–CH₂–NH₂} + \text{H–CHO} \rightarrow \text{R–CH₂–N–CHO}
\]

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The reaction may proceed at RT in FFT and FS. There are, when secondary amines are added to aldehydes, \( N, N \)-disubstituted hemiaminals, \( R_1 \text{CHO} + \text{R}_2\text{R}_3\text{NH} \to R_1\text{CHO}H\text{NH}R_2 \), are formed initially. These hemiaminals cannot lose water in the same way as the primary amines (9); tertiary amines can only give salts:

\[
R_1\text{CHO} + \text{R}_2\text{R}_3\text{R}_4\text{N} \to R_1 - \text{CHO}^- - \text{N}^+\text{R}_2\text{R}_3\text{R}_4
\]

When an aldehyde is treated with a primary or secondary amine in the presence of hydrogen and a hydrogenation catalyst, reductive alklylation takes place. These reactions were not observed in practical samples, but they are possible, if the product(s) are thermodynamically stable in FFT and FS.

### Other reactions: formaldehyde reactions with hydroxides and sulfides

Reactions of formaldehyde with hydroxide and sulfide derivatives together with formic acid may be thought of as similar to the Eschweiler–Clarke reaction:

\[
\begin{align*}
\text{HCHO} + R_1\text{OH} + \text{HCOO}^-\text{H}^+ & \to H_2(\text{OH})\text{C}O^-\text{H}_3 + \text{HCOO}^- \\
& \to H_2C\text{OR}_1 + \text{H}_2O + \text{CO}_2
\end{align*}
\]

\[
\begin{align*}
\text{HCHO} + R_2\text{SH} + \text{HCOO}^-\text{H}^+ & \to H_2(\text{OH})\text{C}S^-\text{H}_3 + \text{HCOO}^- \\
& \to H_2C\text{SR}_1 + \text{H}_2O + \text{CO}_2
\end{align*}
\]

The reaction may proceed at RT in FFT and FS. There are, however, no data and references for the reaction.

### Reactivity of formaldehyde with unsaturated fatty acids—Prins reaction

Reactions between formaldehyde and substances with double bonds are also established according to the Prins reaction as follows:

\[
\begin{align*}
\text{HCHO} + R_2\text{R}_3\text{C} &= \text{CR}_2\text{R}_3 + \text{H}^+ \\
& \to \text{CH}_2(\text{OH}) - \text{CR}_1\text{R}_2 - \text{C}^+\text{R}_3\text{R}_4 \\
\text{CH}_2(\text{OH})\text{C}R_2\text{R}_3 + \text{H}_2\text{O} & \to \text{CH}_4\text{OH} - \text{CR}_1\text{R}_2 - \text{CR}_3\text{R}_4(\text{OH}) + \text{H}^+
\end{align*}
\]

In 1972, Jones (1) described in detail that the reaction of formaldehyde with unsaturated fatty acids produced modified substances. He demonstrated that reactions between a representative substance, oleic acid and formaldehyde yield four major products and four minor products (1). This paper also showed the possibility of a reaction between formaldehyde and peptide bonds, and amino acids with a hydroxide group (serine, threonine and hydroxyproline), aromatic moieties (phenylalanine, tyrosine and tryptophan) and sulfide (cysteine).

### Stability of medications and chemicals in FFT and FS

In general, medicines and chemicals in FFT and FS are relatively stable under acidic conditions, gradually leaching into FS. These substances gradually dilute to a constant ratio determined by the total amount of substances and total volume or mass. Some substances may be partially decomposed by FS. Decomposition generally has a tendency to increase together with pH, the formaldehyde concentration and temperature.

This report describes in detail each substance from the 1970s to May 2012, as summarized in Table I.

### Stability of barbiturates

Regarding barbiturates, six studies were conducted from 1957 to 2011. Sunshine and Hackett (3) reported a study of barbiturates in FFT and FS using an UV technique. Tiess (5) examined barbiturates in FFT using an UV technique. Tsoukali-Papadopoulou (17) described a test for the detection of phenobarbital in brain tissues and FS by UV, thin layer chromatography and gas chromatography (GC) methods.

Winek et al. (18) described the detection of phenobarbital in formalin–blood mixtures (containing 1.9 and 3% formaldehyde) over a 4-week period. Phenobarbital concentrations in formalin-fixed liver(s) (FFL) decreased to \(~50\%\) 1 day after fixation, and 10–17% phenobarbital was detected in FS. They showed the leaching of phenobarbital from the liver into FS. Under their conditions, phenobarbital was relatively stable in a 1.9 or 3% formaldehyde–blood mixture for 4 weeks.

Gannett et al. (19) described experiments where phenobarbital, pentobarbital and secobarbital were used for stability tests under conditions of 5, 10 and 20% FS (1.9, 3.7 and 7.4% formaldehyde, respectively) with and without buffering, using 10 mM potassium phosphate (pH 7 and 9.5). Pentobarbital and secobarbital were stable, but phenobarbital in FS (pH 9.5) partially decomposed to 2-phenyl-butylic acid (at RT). At 30 days after incubation, the phenobarbital level decreased to \(~20\%\) and 2-phenyl-butylic acid increased to \(~50\%\) of the level of the initial phenobarbital. In the conditions described by Gannett and colleagues, phenobarbital decomposition depended on pH and time period.

Cingolani et al. (20) described determinations of phenobarbital and butalbital in FFT and FS with liver. They analyzed these barbiturates by GC–MS using direct on-column methylation after solid-phase extraction. Liver tissues stored in phosphate-buffered 10% FS (pH 7) for 6 months were analyzed. Phenobarbital levels with FFL in four cases averaged 57%, and in FS, 31%. Butalbital levels in FFL from two cases averaged 22%, and in FS, 67%. Their results showed that phenobarbital and butalbital were stable in phosphate-buffered 10% FS (3.7% formaldehyde, pH 7) for 6 months.

Figure 1. The Eschweiler–Clarke conversion. Formaldehyde reacts with primary amines together with formic acid in aqueous solution at room temperature in a multistep process over time.
Barbiturates were stable in FS at acidic and neutral pH, but the decomposition of phenobarbital occurred in buffered FS at pH 9.5. Barbiturates in blood and liver tissues were relatively stable at 4 and 25°C without formaldehyde (21), but there were no data on the influence of pH. Therefore, we need to study whether formalin accelerates the decomposition of barbiturates under alkali conditions.

Stability of tricyclic antidepressants

Winck et al. (18) described the detection of desipramine in FFT and FS using GC. Desipramine in FFL with 1.9% FS 1 day after incubation remained 103% against the control liver specimen, and then 61% (7 days) and 34% (21 days). In 3% formaldehyde-fixed liver, the same tendency was observed. These authors did not refer to imipramine in the report.

Dettling et al. (22) reported the production of amitriptyline (Amp) from Ntp in FS at RT as assayed by GC–MS. They experimentally analyzed Amp and Ntp using nonbuffered 1–40% formaldehyde solutions (pH 4.5) containing Ntp, which were stored for 24 h to 1 week. Ntp decreased to 22% (1.0% formaldehyde), 33% (10%) and 68% (40%). Amp was not present initially, but was subsequently assayed at 1.9% (1.0% formaldehyde), 6.1% (10%) and 47.9% (40%) of the initial level of Ntp. Amp was not detected in 0% FS (a solution without formaldehyde). The same tendency was observed at 48 h, 96 h and 1 week. The authors first showed that N-methyl-Ntp (Amtp) was produced in FS at RT. They also showed that the production of Amp increased more under alkali (pH 9.5), than acidic, conditions (pH 2 or 4). They did not, however, make clear the reason for the phenomenon in the report.

Winic et al. (23) reported that tricyclic antidepressants (TCAs) were detected using FFL and FS together with frozen liver specimens from human autopsy cases by GC and HPLC. Imipramine markedly increased both in FFT and FS in two cases. It was suggested that imipramine was produced from desipramine in FFT and FS.

Takayasu et al. (10) reported an experimental study using TCAs in various FS at RT (15–25°C) by GC–MS in 1998. It was shown that Ntp was converted into Amp and desipramine into imipramine in FS, especially under alkali conditions. They first suggested that the imipramine and Amp in FS were produced from desipramine and Ntp, respectively, by the Schewieler–Clarke conversion (7–9).

Stability of methamphetamine and MDMA

Takayasu et al. (24) reported that MA was determined by GC–MS using FFT in rabbits for 1–28 days at RT (10–20°C). In liver tissue, MA decreased to 2.3% (1 day), 3.2% (7 days), 1.0% (14 days) and 0.2% (28 days) compared with before fixation. Other organs, the brain, lungs, kidneys and skeletal muscles, showed the same tendency. It was suggested that MA reacted nucleophilically with formaldehyde to form additional products. They did not, however, detect N-methyl-MA (M-MA), or refer to the Schewieler–Clarke conversion.

Tirumalai et al. (12) reported the results after MA was mixed with phosphate-buffered 5, 10 and 20% formaldehyde solutions (pH 3.5, 7.0 and 9.5) for 1–30 days. MA was determined by HPLC. In the pH 9.5 10% formaldehyde solution, MA decreased to ~30% (1 day) and 0% (7 and 30 days), whereas N,N-dimethylamphetamine (M-MA) increased to ~60% (1 day), 95% (7 days) and 97% (30 days). At pH 3.5, MA was almost constant over 30 days, but little M-MA was detected at any time. M-MA was identified by GC–MS–MS in the liver. The conversion from MA to M-MA was explained by the Schewieler–Clarke reaction.

Shakleya et al. (16) described human liver tissue with MDMA HCl, placed it into 20% nonbuffered FS (pH 5.5–5.7) and then vortex mixed it. After 24 h incubation at RT, an aliquot of the supernatant was mixed with 0.1% formic acid in acetonitrile and then directly analyzed by ESI–MS–MS. N-Methyl-MDMA (MDMA) was detected by ESI–MS–MS. It is, however, possible that M-MDMA was produced in the ionization port, because MDMA, formaldehyde and formic acid in the sample solution were mixed and heated at the ESI port. It is not clear whether M-MDMA was produced in the FS or ESI port [e.g., LC–MS(–MS) after extraction could be used for solution].

Shakleya et al. (13) performed an experiment with MA using almost the same method with MDMA (16). The frozen liver tissues from four autopsy cases were used. The sample was directly applied to ESI–MS–MS, and MDMA was determined. Although the possibility of methylation of MA in FS exists, the mechanism should be verified by other methods (e.g., LC–MS–MS after extraction).

Table I.
List of references relation to the determination of substances in FFT, FS and embalmed cases

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Target substances</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1957</td>
<td>Sunshine and Hackett</td>
<td>Barbiturates</td>
<td>(3)</td>
</tr>
<tr>
<td>1967</td>
<td>Tieiss</td>
<td>Barbiturates, methaqualone, glutethimide, chloroquine, nicotine</td>
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<td>Tricyclic antidepressants</td>
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<td>1993</td>
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<td>1994</td>
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<td>2004</td>
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<td>2005</td>
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<td>Phenoxybarbital, butalbital</td>
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<td>2006</td>
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<td>Bupropion, olanzapine</td>
<td>(25)</td>
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<td>Suma and Sai Prakash</td>
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<td>2008</td>
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<td>2010</td>
<td>Miyaguchi and Sekine</td>
<td>Aconitum alkaloids</td>
<td>(32)</td>
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In summary, data from the described studies show that MA is not stable in FS or FFT. MDMA and MA both appear to be methylated in the presence of formaldehyde; however, the experimental data need additional clarification to distinguish between a true reaction and an analytical artifact.

**Stability of fenfluramine, sertraline, fluoxetine, bupropion and olanzapine**

Gannett et al. (11) carried out experiments on FF with 5, 10 and 20% formaldehyde solutions (nonbuffered, 10 mM phosphate-buffered at pH 7 and 9.5) for 1–30 days by HPLC. N-methyl-FF (M-FF) was produced at pH 7 and 9.5, but not in nonbuffered acidic FS. The amount produced was dependent on pH and the concentration of formaldehyde. They explained the conversion from FF to M-FF by the Eschweiler–Clarke reaction.

Suma and Sai Prakash (14) reported that Srt was incubated with FS (5, 10 and 20%) with phosphate-buffered (pH 3.0, 7.0 and 9.5) at RT (≏25 C) for 4–30 days and then analyzed by GC–MS after extraction with chloroform. In 10% FS, Srt decreased to 20–40% (pH 3.0), 10–40% (pH 7.0) and 0–40% (pH 9.5), and N-methyl-Srt (M-Srt) increased to 20–70% (pH 3.0), 10–70% (pH 7.0) and 20–100% (pH 9.5) against the initial Srt concentration. In 20% formaldehyde, the tendency was marked. According to these results, M-Srt production was dependent on pH and the concentration of formaldehyde. They detected an imine intermediate of Srt that disappeared after 14 days. A mass spectrum of the imine was recorded, but the data were not included in detail. The imine was thought to be a Schiff base. The conversion from Srt to M-Srt was explained by the Eschweiler–Clarke reaction. If the imine intermediate was not a false positive, this may be the first data for both a Schiff base and the Eschweiler–Clarke conversion.

Suma et al. (15) reported that Flx was injected into a piece of rat liver. The liver was then immersed with 5–20% of FS at three pH levels (3.0, 7.0 and 9.5) and a control and held at RT (≏25 C) for 0–30 days. The homogenized specimens were centrifuged. An aliquot of the supernatant with methanol added was directly injected into GC–MS. At pH 3.0 for 0 days, Flx remained ≏50% (5% FS) and 30% (20%), whereas N-methyl-Flx (M-Flx) was ≏50% (5% FS) and 70% (20%). Because the homogenized sample solution contained formalin (formaldehyde and formic acid as contaminant) and methanol was directly applied into the GC-injection port, it was not clear whether M-Flx production occurred in FS or in the GC-injection port.

Suma et al. (25) described bupropion (an antidepressant) stored in the same conditions as those used by Suma and Sai Prakash (14) that reacted with formalin and formic acid to form N-methylbupropion by the Eschweiler–Clarke conversion. The reactivity was greater at pH 9.5 than pH 3.0 and with 20% formalin than with 5%. Olanzapine, however, reacted to form only 2% N-methyl derivative that was observed at the end of the stability study. Therefore, it may be concluded that the secondary amine in the seven-membered ring of olanzapine had less reactivity than bupropion, which is an aliphatic secondary amine.

**Stability of benzodiazepines**

Winek et al. (18) described the detection of diazepam by GC in FFT and FS using liver. They used experimentally mixed blood containing diazepam and FS (1.9 and 3% formaldehyde). Diazepam was determined by GC–ECD after extraction with diethyl ether. Diazepam decreased to ≏41% (1.9% formaldehyde) and ≏56% (3%) 31 days after incubation, while control whole blood decreased to 88% when compared with the initial concentration.

Nishigami et al. (26) injected diazepam into rabbits and then collected tissue samples and used them for three kinds of FS [nonbuffered 10% (pH 5.1), buffered 10% (pH 7.4) and buffered 4% paraformaldehyde (pH 7.4)] over 28 days at RT. In the lung and skeletal muscle samples immersed in 10% buffered formalin (pH 7.4), diazepam concentrations tended to increase. In all samples, diazepam was also detected over 28 days (10–70% in three kinds of FS).

Tracy et al. (27) reported that 10 benzodiazepines (alprazolam, clorazepoxide, diazepam, flunitrazepam, flurazepam, lorazepam, midazolam, oxazepam, prazepam and triazolam) were mixed with 5, 10 or 20% FS (pH not adjusted, pH 7 and pH 9.5 with phosphate buffer). Samples were directly analyzed by HPLC 1–30 days after incubation. Alprazolam, midazolam, triazolam and prazepam were labile in acidic conditions with formaldehyde, and flunitrazepam, lorazepam and flurazepam were labile in alkaline conditions. Chlordiazepoxide had sensitivity in both, but oxazepam showed no change in the degradation rate against the control. These benzodiazepines, except for alprazolam, midazolam and chlordiazepoxide, were relatively stable in FS.

The stability of benzodiazepines under various conditions without formalin was reported by other authors (28, 29).

**Stability of cocaine and aconitum alkaloids**

Cingolani et al. (30) described a study of cocaine and its metabolite benzoylecgonine (BE) in fresh liver tissues, FFL stored for 4 weeks, and FS (10%, pH 7). Analysis was determined by GC–MS after solid-phase extraction in four autopsy cases. In one of four cases, cocaine was detected in the fresh liver tissue, but not in FFL or FS. In three cases, only BE was determined in the three kinds of samples; cocaine was not detected. Recoveries of BE in both FFL and FS averaged 97% (75–118%) compared with those in fresh liver tissue. According to these results, the cocaine metabolite BE was relatively stable, but cocaine was labile.

Viel et al. (31) reported that cocaine and BE were determined in liver and brain tissue from an autopsy case, and these tissues were fixed using FS at pH 3.5 (nonbuffered) and pH 7.4 (buffered) for 30 days at RT. Cocaine and BE in FFT decreased to ≏45–50% in the brain and ≏20% in the liver at both pH values. Cocaine was relatively stable at pH 3.5, but less stable at pH 7.4, and BE was stable in both conditions. These results are not inconsistent because cocaine has an ester structure that is labile in alkali conditions.

Miyaguchi and Sekine (32) reported that benzoylecgonine and benzyolmesaconine were detected by LC–MS–MS in FFT (liver, kidney and lung) from a homicide case. Concentrations (ng/g) of benzyolmesaconine were 0.26 (liver), 0.22 (kidney) and 0.20 (lung), but those of benzoylecgonine were <0.1 ng/g. They described the absence of aconitine or mesaconitine as a result of most of the diester-type alkaloids being hydrolyzed to monoesters, and these were eluted to FS from FFT. They also indicated that the degradation of diester-type alkaloids, aconitine or mesaconitine was due to four factors: antemortem metabolism,
postmortem changes by endogenous or exogenous enzymes, nonenzymatic hydrolysis in FS and reactions with formaldehyde.

**Stability of methadone, propoxyphene, fentanyl, morphine and heroin**

Franssioll et al. (33) reported that methadone concentrations in the brain and liver fixed 14 months after formalin-incubation increased compared with those in frozen tissues, but concentrations in the kidney and lung decreased. It was further described that propoxyphene was determined in six other cases 4–24 months after fixation. Methadone and propoxyphene were determined in FFT in cases by comparing both the GC and enzyme multiplied immunoassay technique (EMIT) methods. Concentrations of methadone and propoxyphene in the FFT changed to both increase and decrease in each tissue compared with the frozen tissues.

Rohrig (34) reported a case involving fentanyl and nine other medications in which fentanyl concentrations in embalmed liver tissue decreased to ~74%. This paper reported that fentanyl concentrations did not increase because of the embalming and burial process.

Cingolani et al. (35) reported five autopsy cases involving morphine. The morphine in FFT was acid hydrolyzed and then extracted with solid-phase extraction columns, and derivatized with N-methyl-N-trimethylsilylt trifluoroacetamide (MSTFA). Morphine concentrations in FFT and FS were similar to those in tissues at autopsy. They described that morphine had good stability in FFT and FS.

Xiang et al. (36) injected morphine intravenously into two rabbits. Some organ tissues were collected and then immersed in FS for 4 months. Morphine was extracted from the tissues after acid hydrolysis, with acetic anhydride derivatization and GC–MS analysis. Morphine was detected in all FFT (0.5–300% compared with frozen tissues), and concentrations were lower in FS than in FFT. They reported that morphine had good stability in FFT and FS.

Alunni-Perret et al. (37) reported an autopsy case in which a man who died in Thailand was repatriated to France after embalming and storage for 6 months. It showed the possibility of determination of succinylcholine in FFT.

Winek et al. (41) described a case where a female who had a long history of medical problems with drug abuse including Placidyl (ethchlorvynol) was autopsied 52 h after embalming. Ethchlorvynol concentrations were determined by GC–FID and measured 112 µg/mL in bile. It showed the utility of bile specimens in embalmed cases.

**Stability of succinylcholine, etchchlorvynol, phenytoin, strychnine, tetramine and lidocaine**

Forney et al. (40) described an experimental study of succinylcholine using embalmed rats. They extracted succinylcholine with hexanitrodiphenylamine and then carried out its demethylation with sodium benzenethiolate. Succinylcholine was determined by GC–MS using the liver, kidney and muscle specimens at the injection sites of rats after embalming and storage for 6 months. It showed the possibility of determination of succinylcholine in FFT.

Winek et al. (41) described a case where a female who had a long history of medical problems with drug abuse including Placidyl (ethchlorvynol) was autopsied 52 h after embalming. Ethchlorvynol concentrations were determined by GC–FID and measured 112 µg/mL in bile. It showed the utility of bile specimens in embalmed cases.

Cingolani et al. (42) described a study of strychnine with FFT and FS in a poisoning case in which liver and kidney specimens were fixed with 10% buffered formalin (pH 7) for 8 weeks and then analyzed by GC–MS after chloroform extraction. The strychnine concentration was 1.6 mg/kg (24%) in FFT and 1.8 mg/L in FS (liver), and 0.98 mg/kg (37%) in FFT kidney and 1.1 mg/L in FS (kidney). They showed that strychnine was relatively stable in FFT and FS.

Xiang et al. (37) reported that tetramine in FFT was determined in one experiment. Tetramine in FS was detected in only two of six samples. Tetramine has a structure, \( \text{[=}\text{N–SO}_2\text{–N=}\] \), which may be sensitive to FS and therefore, may be relatively unstable in FS.

Kudo et al. (43) reported a case of poisoning in which lidocaine in FFT was determined by GC–MS 40 days after fixation: brain 0.22–0.32 µg/g; liver 0.11 µg/g; kidney 0.052 µg/g; skeletal muscles 0.13 µg/g and FS (8.4 ng/g). An experiment with rats using 10% formalin (pH 7) also showed that lidocaine concentrations in FFT decreased by one-third to one-fourth.

**Stability of paraquat and malathion**

Kuo and Kuo (44) described a paraquat determination method using sulfuric acid treatment and cation exchange for a clean-up.
technique. Paraquat concentrations were determined using FFT in two autopsy cases by UV spectrometry. In one case, the paraquat concentration (mg/kg) was 0.65 in the liver and 1.33 in the kidney. In the other case, it was 0.88 in the liver and 1.01 in the kidney.

Tanaka et al. (45) described an embalmed autopsy case with tissues stored for 44 days at RT. The aequous fixation solution contained 5% formalin (2% formaldehyde), 26% ethanol, 5% phenol and 10% glycerine (v/v). The postmortem malathion concentrations (μg/mL or μg/g) in the decedent’s body fluids and FFT determined by GC–MS were as follows: 1.9 (heart blood), 0.35 (stomach contents), 0.38 (cerebrum), 5.3 (heart muscle) and 0.21 (liver). It showed the possibility of a quantitative analysis of malathion in FFT.

**Stability of cyanide, carbon monoxide and carbon monoxide-hemoglobin**

Winck et al. (18) described an attempt to detect cyanide in formalin–blood mixtures (1.9 and 3% formaldehyde) over 4 weeks. Despite using a classic microdiffusion technique, they could not detect cyanide.

Iffland et al. (46) reported carbon monoxide (CO)–hemoglobin (Hb) contents in clots of blood in an embalmed corpse. They developed a method whereby CO–Hb levels in blood clots were calculated from both CO contents in a sample by head-space GC using a hydration column of CO and Hb contents deduced from iron (Fe) contents in the same sample. The method was first used with liquid nitrogen for sample freezing and protection of CO loss from the sample. Fe content in the clots was measured by atomic absorption spectrometry after dissolving with a 70% nitric acid solution. Hb contents were then deduced from Fe contents. The method was preferable for altered blood (e.g., putrefied, heated and formalin-fixed), but was expensive and time-consuming compared with an automatic CO-oximeter.

Middleberg et al. (47) determined CO–Hb contents in FFT (spleen and muscle) by the combined method of head-space GC–MS (CO gas) and flame atomic absorption spectrophotometry (Fe content). The authors showed that volatiles could be detected in FFT at least within 14 days.

**Conclusions**

In this review, the mechanisms of formaldehyde reactions to substances were explained as the Prins reaction, Schiff base formation and Eschweiler–Clarke conversion in FFT and FS. The methylation of substances including primary and secondary amines by Eschweiler–Clarke reaction could, in particular, occasionally influence the estimation of the concentrations of substances in FFT, which would have an impact on forensic diagnosis and toxicology. In addition, this review references studies between the 1970s and 2012 concerning the determination of substances in FFT and FS. It is hoped that this review will support the development of new approaches for the determination of substances in FFT and FS and embalmed autopsy cases.

**References**


