

Review

Clinical and Post Mortem Aspects of Methomyl Intoxication

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Summary

Methomyl is a carbamate pesticide that induces acute cholinergic poisoning in mammals by reversible inhibiting acetylcholinesterase activity. In the Netherlands, methomyl is not legally registered for use as an active substance in pesticides. However, intoxications with non-registered pesticides have been reported before. The goal of this review, therefore, is to summarize clinical and post-mortem aspects that should be considered in forensic investigations of suspected methomyl intoxications.

In clinical and post mortem situations the inhibition of (acetyl)cholinesterase activity is used as a biomarker in methomyl intoxications and methomyl is analyzed in biological samples. In clinical settings the kinetic pattern of the acetylcholinesterase enzyme activity can be used to differentiate between carbamates and organophosphates. Post mortem concentrations of methomyl are probably highest in cerebrospinal fluid and vitreous humor. Hairs might be used to detect past exposure to methomyl.

However the inhibition of (acetyl)cholinesterase by carbamates is reversible and post mortem reactions decrease the concentrations of methomyl in biological samples. Therefore, further research in humans is needed on probable alternative analytical markers like methomyl metabolites and factors associated with oxidative stress caused by methomyl intoxication. Additionally, data on possible relationships between amount of methomyl ingested, methomyl concentration in blood, and blood (acetyl)cholinesterase activities are lacking.

1. Introduction

Methomyl is a carbamate pesticide, marketed as an aqueous solution and in solid form with brand names like Lannate, Nudrin, Metomex, and Terlate¹. Products containing methomyl have a broad spectrum of application from commercial baits to professional concentrates. It inhibits acetylcholinesterase activity that results in increased acetylcholine levels. Consequently disrupting the normal nerve synaptic function, affecting mainly the peripheral nervous system². Acute toxicity studies of methomyl in animals showed that this carbamate was highly toxic orally, when given acutely. However, methomyl had a low order of chronic oral toxicity due to rapid metabolism and elimination³. Apart from the poisoning caused by gastrointestinal absorption the high potential of methomyl poisoning through inhalation or transdermal absorption has also been reported⁴. Additionally, methomyl was highly toxic via ocular exposure⁵.

In Europe, pesticides may only be used and handled if they are legally registered. In the Netherlands, methomyl is not legally registered for use as an active substance in pesticides⁶. According to the Pesticides Database from the Dutch Board for the Authorisation of Plant Protection Products and Biocides (2010)⁶, a total of 16 pesticides with methomyl as the active substance were retrieved from the Dutch market. Expiry dates started from September 1995 up to February 2009. Since some of these retrievals were quite recent, accidental or deliberate intoxications might occur by exposure to methomyl left behind at sites from former users of methomyl pesticides. Belgium and Germany are countries closest to the Netherlands. In Belgium no products are registered with methomyl as active substance. One product containing methomyl (Lannate 20 SL) was retrieved from the Belgium market in March 2008. This product had an expiry date in March 2009⁷. Germany has no methomyl-containing products on the list of authorized plant-protection products for the German market. In Germany, methomyl-containing products are also not present on the terminated authorization list or the suspended authorization list for plant-protection products⁸. There are however, countries where the use of methomyl as a plant-protection product is legally registered, among which: Australia, Canada, India, Madagascar, New Zealand, Philippines, South Africa, United Kingdom, United States, and Vietnam⁹. It may be possible that methomyl containing products incidentally enter the Netherlands illegally from a foreign country. So, methomyl intoxication in the Netherlands might still occur due to exposure to methomyl containing products from native left-overs or foreign sources.

The Dutch Forensic Institute requires toxicological data on various intoxications. Up till now, summarized information on clinical and post mortem aspects of methomyl intoxication was hardly available. The goal of this review is to summarize clinical and post-mortem aspects that should be considered in forensic investigations of suspected methomyl intoxications.

2. Methomyl

2.1. Chemical properties

Methomyl, S-methyl N-((methylcarbamoyl)oxy) thioacetimidate (Figure 1), (CAS no 16752-77-5) belongs to the chemical family of the carbamates. Pure methomyl is a white crystalline solid, with a slightly sulfurous odor, a melting point of 78-79°C, and a molecular weight of 162.2. Methomyl is soluble in water (5.8g/100g) and in most organic solvents. Methomyl decomposes slowly in water. The rate of degradation increases with temperature, alkalinity, aeration, and sunlight.⁵

In general, carbamates are rapidly metabolized and excreted^{10, 11}. However, precise kinetics of methomyl metabolism in humans are unknown¹¹. According to the World Health Organization (1983)¹², methomyl is non-cumulative and rapidly metabolized in plants and animals to substances of lower toxicity.

Methomyl is also a degradation product of the carbamate thiodicarb (CAS no 59669-26-0)^{5, 13}. This carbamate is also not registered in the Netherlands and its neighbouring countries. Thiodicarb consists of two methomyl moieties, joined together by their amino nitrogen by sulfur (Figure 1). After oral ingestion in rats thiodicarb was rapidly degraded in the stomach to its methomyl derivative, and few other unstable intermediates. The activity of thiodicarb is closely related to that of methomyl¹³.

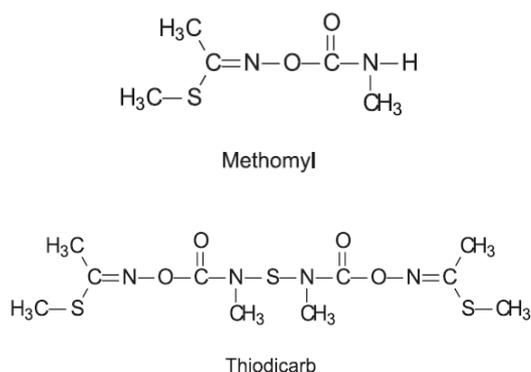


Figure 1: Chemical structures of methomyl and thiodicarb¹³

2.2. Application

According to the U.S. Environmental Protection Agency (1998)⁵, methomyl is registered on a wide variety of sites. An estimated 2.5 to 3.5 million pounds active ingredient of methomyl are applied annually in the U.S.. Methomyl is used for foliar treatment of vegetable, fruit and field crops, cotton, commercial ornamentals, and in and around poultry houses and dairies. It is also used as fly bait. Methomyl is highly effective for control of over 100 species of insects as an ovicide, as a larvicide, and as an adulticide¹⁴. It is also used as an acaricide to control ticks and spiders⁵.

Methomyl is an anti-cholinesterase agent and belongs to the chemical family of the carbamates. Anti-cholinesterase pesticides are subdivided into two main groups: organophosphates and carbamates. At first, organophosphates were more widely used. Later on, carbamates rapidly gained acceptance due to their similar efficacy in many crops but with a relatively smaller risk of toxicity in humans compared to organophosphates¹⁵, (paragraph 3.3).

According to the U.S. Environmental Protection Agency (1998)⁵, laboratory studies indicated that methomyl is moderately persistent and highly mobile. Methomyl was stable to hydrolysis at lower pH's (neutral to acidic), and degraded slowly in alkaline conditions. Methomyl photolyzed quickly in water, but more slowly in soils. It was moderately stable to aerobic soil metabolism, but degrades more rapidly under anaerobic conditions. In laboratory studies, methomyl did not readily adsorb to soil and had the potential to be very mobile.

In the Netherlands, methomyl is not legally registered for use as an active substance in pesticides. A total of 16 pesticides with methomyl as the active substance were retrieved from the Dutch market, with expiry dates up to February 2009⁶. Still, intoxications with methomyl may occur. This was also demonstrated in a case of a fatal thiodicarb poisoning in France in 2008. This pesticide is no longer registered in France, but was still stored and deliberately ingested¹³.

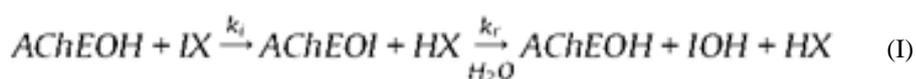
2.3. Mechanism of toxicity

Acute toxicity

Carbamates like methomyl inhibit acetylcholinesterase. Under normal conditions, acetylcholinesterase rapidly and efficiently hydrolyzes acetylcholine in the synaptic cleft of cholinergic neurons (Figure 2). Cholinergic neurons synthesize acetylcholine from choline and the cofactor acetyl coenzyme A through the action of the synthetic enzyme choline acetyltransferase. The acetylcholine molecules are packaged into synaptic vesicles by the vesicular acetylcholine transporter. Upon depolarization of the nerve terminal synaptic vesicles fuse with the plasma membrane, and release acetylcholine into the synaptic cleft. Prior to inactivation by acetylcholinesterase, acetylcholine molecules interact with postsynaptic cholinergic receptors to propagate the nerve pulse, and/or to alter cellular function (excitation). The cellular function is altered either by altering ion flux across the postsynaptic cell membrane, or through the generation of intracellular second messengers. When cholinesterase inhibitors, like methomyl, bind to a substantial number of acetylcholinesterase molecules, the degradation of acetylcholine is prevented and transmitter molecules accumulate in the synapse. Elevated synaptic acetylcholine levels lead to persistent stimulation of cholinergic receptors on postsynaptic cells and subsequent alteration of cholinergic receptor-mediated signaling pathways (overexcitation), e.g. alteration of intracellular cAMP levels.¹⁶ Due to the inhibition of acetylcholinesterase, parasympathetic autonomic postganglionic nerve fibers (which contain muscarinic receptors) and somatic motor nerve fibers (which contain nicotinic receptors) become overstimulated¹⁷.

In the parasympathetic nervous system, excessive cholinergic stimulation has effect on nicotinic receptors at the parasympathetic ganglia and on muscarinic receptors at various exocrine glands and hollow smooth muscle viscera. These effects result in diverse actions as: bladder and bowel stimulation; sphincter relaxation; miosis; bronchoconstriction; and increased secretions from the lacrimal, salivary, nasopharyngeal, pancreatic, and bronchial glands. Stimulation of the vagus nerve to the heart can result in bradycardia. In the sympathetic nervous system, excessive cholinergic stimulation of nicotinic receptors at the sympathetic ganglia may result in catecholamine release and some sympathetic effects. However, these sympathetic effects are typically overwhelmed by the effects on the parasympathetic nervous system. Excessive cholinergic stimulation of muscarinic receptors at postganglionic neurons from the sympathetic nervous system results in stimulation of sweat glands. At the neuromuscular junction overstimulation of nicotinic receptors on the motor endplate causes myocyte depolarization. Thereafter, repolarization is inhibited and the muscles can no longer contract.¹⁸ All these effects lead to symptoms that are part of the cholinergic syndrome discussed in chapter 3.

The inhibition of acetylcholinesterase by carbamates is reversible, and can be described by the following enzyme-substrate reaction:



The active site on AChE contains a serine residue (AChEOH). An anti-cholinesterase agent (IX) can bind to the active site. For a carbamate this process is called “carbamylation”. The serine hydroxyl group gives up its hydrogen to the X group on the carbamate molecule which then splits off as HX. Thereby, the esteric site of AChE is occupied (AChEOI). This prevents acetylcholine from interacting with the cholinesterase enzyme and being broken down. The carbamylation of the active enzyme takes place with rate k_i .

The inhibited enzyme may be reactivated by hydrolysis of the carbamyl residue (IOH) with reactivation rate k_r leaving the inactivated inhibitor^{18, 19}. The inhibition is a second order process, the rate of which depends on the concentration of the carbamate and the active enzyme. Reactivation is a first order spontaneous process due to the abundance of water in which the inhibited enzyme can be reactivated rapidly with a half-time of an hour or less for carbamates^{19, 20}.

Toxicity of *N*-methyl carbamate pesticides like methomyl manifests itself rapidly, usually within an hour after dosing. Bioactivation of these pesticides is not required²¹. Carbamates are rapidly metabolized because of their easy degradation and lack of chemical stability. Their elimination is also fast¹¹. Carbamates are well absorbed across the lung, mucous membranes (including gut), and skin. Significant toxicity has been reported after all these routes of exposure²². Acute toxicity studies of methomyl in animals showed that this carbamate is highly toxic when administered orally. However, due to rapid metabolism, methomyl has a low order of chronic oral toxicity³. According to the European Food Safety Authority (2006)²³, methomyl is highly toxic via the oral, ocular, and inhalation routes of exposure, but it has a low toxicity via the dermal route. However, some cases of fatal poisoning by methomyl caused by transdermal absorption have been reported^{4, 24, 25}. Methomyl is not an eye or skin irritant, and does not cause skin sensitization²³.

Chronic toxicity

According to the World Health Organization (1983)¹², methomyl is rapidly degraded in organisms and the environment. Therefore, chronic toxicity is not expected to be a major problem. According to the U.S. Environmental Protection Agency (1998)⁵, available chronic toxicity and carcinogenicity studies demonstrated no evidence of carcinogenicity. They stated that based on available studies the weight of the evidence indicated that methomyl did not pose a genotoxic, reproductive, or developmental concern. But, in addition to its anti-cholinesterase activity methomyl may induce oxidative stress as indicated by studies in mice²⁶ and rats^{27, 28}. In these animals, levels and activities of antioxidant defense system enzymes (e.g. glutathione-S-transferase and superoxide dismutase) and lipid peroxidation were altered due to methomyl intoxication. Pesticides like methomyl have shown to cause overproduction of reactive oxygen species. When reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms oxidative stress and potential tissue damage results^{27, 28}.

Metabolism

The U.S. Environmental Protection Agency (1998)⁵ states there are two main animal metabolites of methomyl, acetamide and acetonitrile. A proposed metabolic pathway for methomyl in animals is shown in Figure 3²⁹. The conversion rate of methomyl to acetamide is low, therefore, residue levels of acetamide will be low. Acetonitrile is volatile and is mostly respired or excreted leaving only small residues. In rats, elimination of methomyl was tracked after oral administration of radiolabeled methomyl. After 1 day 10% of the methomyl was recovered in the whole body. The radiolabel eliminated (90%) was detected in respiratory CO₂ and urine, the ratio being 1 part [¹⁴C]CO₂, 2 parts [¹⁴C]acetonitrile, and 1 part urinary metabolites³⁰. One urinary metabolite was examined in another rat study. The elemental composition of this urinary metabolite was found to be C₅H₁₀N₂O₅S₂¹⁴. The metabolites S-methyl-N-hydroxy-thioacetimidate and S,S'-dioxide-methomyl were expected, but were not found in these studies¹. According to Reiser *et al.* (1997)¹⁴, isolation and purification of the highly polar metabolites was difficult because their physical and chemical properties are similar to those of many natural products.

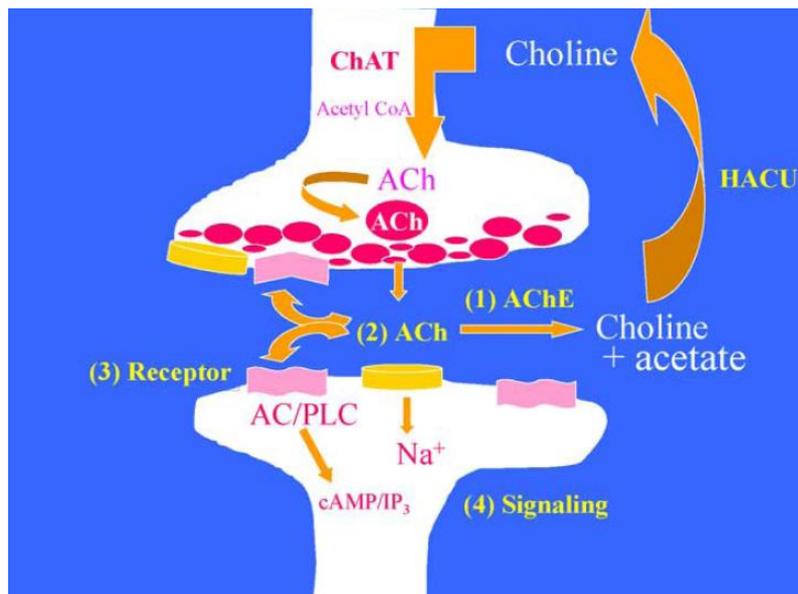


Figure 2: The cholinergic synapse and cholinesterase inhibitors. Normally acetylcholinesterase (AChE) efficiently hydrolyzes acetylcholine released by the presynaptic terminal. The choline released is then transported back into the presynaptic terminal, by the high affinity choline uptake (HACU) process. Acetylcholine (ACh) is formed by the synthetic enzyme choline acetyltransferase (ChAT), using choline and acetyl coenzyme A. ACh is concentrated in synaptic vesicles by the vesicular acetylcholine transporter. Upon depolarization of the nerve terminal, synaptic vesicles fuse with the plasma membrane, and release ACh in the synaptic cleft. When sufficient cholinesterase inhibitors bind to AChE to (1) block transmitter degradation, ACh accumulates (2) in the synaptic cleft. This leads to persistent stimulation of cholinergic receptors (3) on the postsynaptic cell. The excessive activation of these postsynaptic receptors leads to prolonged cholinergic receptor signaling (4) and associated changes in postsynaptic cell function¹⁶.

2.4. Exposure limitsⁱ

For methomyl several values have been determined from animal studies and cases of methomyl intoxication in humans. The Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL), and the Acute Reference Dose (ARfD) for methomyl were set at 0.0025 milligram per kilogram bodyweight (mg/kg bw)²³. This value was based on the acute neurotoxicity No Observed Adverse Effect Level (NOAEL) of 0.25 mg/kg bw, found in rats³¹. A Safety Factor (SF) of 100 was used to determine that the ADI, AOEL, and ARfD for humans is 0.0025 mg/kg bw. The TD₅₀ for humans was calculated by Buchholz *et al.* (2002)³². Their study was based on an outbreak of food-borne illness associated with methomyl-contaminated salt. The oral TD₅₀ is the toxic dose causing symptoms in 50% of those exposed. Buchholz *et al.* (2002)³² estimated the oral TD₅₀ for humans to be 0.15 mg/kg bw with an estimated range of 0.09-0.31 mg/kg bw.

The oral LD₅₀, is the lethal dose in 50% of those exposed. Kaplan *et al.* (1977)³ determined the oral LD₅₀ in male rats to be 17 mg/kg bw. Al-Baggou *et al.* (1999)³³ determined that intraperitoneal administration of methomyl led to a 24h LD₅₀ in rats of 6.29 mg/kg. In humans, the lethal dose of methomyl is estimated to be 12-15 mg/kg bw, based on three fatalities caused by accidental methomyl poisoning¹⁰. Miyazaki *et al.* (1989)³⁴ estimated the lethal blood concentration of methomyl in humans to be higher than 1 mg/kg, based on a case of a fatal methomyl intoxication. Results from a study of Tsatsakis *et al.* (1996)¹¹ implied that a fatal outcome might be associated with blood methomyl concentrations in humans of 3.24-28.0 mg/L.

ⁱ ADI= Acceptable Daily Intake, a measure of the amount of a substance in food or drinking water that can be ingested over a lifetime without an appreciable health risk.

AOEL= Acceptable Operator Exposure Level, the maximum amount of active substance to which the operator may be exposed without any adverse health effects.

ARfD= Acute Reference Dose, an estimate of the amount of a substance in food or drinking water, normally expressed on a body weight basis that can be ingested in a period of 24 hours or less without appreciable health risks to the consumer on the basis of all known facts at the time of the evaluation.

NOAEL= No Observed Adverse Effect Level, the level of exposure of an organism found by experiment or observation at which there is no biologically or statistically significant increase in the frequency or severity of any adverse effects in the exposed population when compared to its appropriate control.

SF = Safety Factor, value used in extrapolation from experimental animals to man, and/or from selected individuals to the general population.

3. Clinical manifestations

3.1. Cholinergic syndrome

Methomyl is a pesticide that inhibits acetylcholinesterase. This leads to symptoms that are part of the cholinergic syndrome. Clinical manifestations of a poisoning with a carbamate, like methomyl, are summarized in Table 1. The mnemonics SLUDGE/BBB, or DUMBELS are used to remember the classic cholinergic symptoms:

SLUDGE/BBB:

Salivation, Lacrimation, Urination, Defecation, GI symptoms, Emesis, Bronchorrhea, Bronchospasm and Bradycardia,

or

DUMBELS:

Diarrhea and diaphoresis, Urination, Miosis, Bronchorrhea, bronchospasm, and bradycardia, Emesis, Lacrimation and Salivation.

The onset of clinical effects subsequent to carbamate exposure depends on: the dose, route of exposure, type of carbamate involved, use of protective clothing and equipment, and the premorbid state of the victim. Ingestion or inhalation of carbamates results in a more rapid onset of clinical effects as compared to dermal exposure³⁵.

In a study by Tsai *et al.* (2003)³⁶ an outbreak of a food-borne illness due to methomyl contamination was investigated. Symptoms reported in 55 cases are shown in Table 2 together with the median latency to onset. Tsai *et al.* divided the severity of cases into three categories (mild, moderate and severe) on the basis of cardiac and respiratory signs.

Mild intoxication

According to Tsai *et al.* (2003)³⁶, a mild toxicity consisted of no respiratory difficulty, bradycardia (heart rate less than 60 beats/min), or ventricular arrhythmia. Buchholz *et al.*³² investigated an outbreak of food-borne illness associated with methomyl-contaminated salt. Although, several persons were treated at emergency departments no one was hospitalized and ill persons recovered in less than 1 day. Symptoms reported for these mild intoxications in 107 cases are shown in Table 2. This outbreak indicated that persons who ingested low levels of methomyl and other carbamates in food may present with symptoms that resemble mild gastrointestinal illness caused by bacterial or viral pathogens. Dizziness and an early onset of diarrhea were indicators of a toxic origin of the poisoning rather than an infectious agents. According to Buchholz *et al.* (2002)³², reports of salivation, even if infrequent, should raise suspicion of potential intoxication with a cholinesterase inhibitor. Tsai *et al.* (2003)³⁶ stated that there are clinical similarities between paralytic shellfish poisoning and cholinergic poisoning. They therefore suggested that acetylcholinesterase activity should be measured in cases with clinical findings similar to paralytic shellfish poisoning to distinguish these kinds of poisonings.

Moderate and severe intoxications

According to Tsai *et al.* (2003)³⁶, moderate toxicity consisted of bradycardia and dyspnea without respiratory failure. Severe toxicity was defined by Tsai *et al.* as respiratory failure requiring endotracheal intubation with or without bradycardia or ventricular arrhythmia. Severe intoxications with methomyl can lead to death. Respiratory failure through a combination of excessive airway secretions, paralysis of muscles of ventilation, and depression of the respiratory control centers in de pons-medulla are generally considered the primary cause of death following lethal methomyl exposures¹⁶.

Table 1: Clinical manifestation of carbamate versus organophosphate poisoning³⁵

	Carbamate Intoxication	OP intoxication
Mucarinic signs	miosis, salivation, sweating, lacrimation, rhinorrhea, abdominal cramping, vomiting, diarrhea, urinary incontinence, bronchospasm, dyspnea, hypoxemia, bradycardia, bronchial secretions, pulmonary edema and respiratory failure.	as in carbamate poisoning
Nicotinic signs	<i>less frequent:</i> muscular twitching, fasciculations, muscle weakens including the respiratory muscles, paralysis, tachycardia, hypertension	<i>common:</i> as in carbamate poisoning
Central nervous system (CNS) signs	<i>rare</i>	<i>common:</i> agitation, confusion, seizures, coma, respiratory arrest
Laboratory findings	AChE inhibition noticed hours after intoxication	AChE inhibition may be prominent weeks after intoxication
Delayed symptoms	<i>rare</i>	intermediate syndrome, delayed neuropathy or neuropsychiatric effects are common
Pediatric population	miosis less prominent; frequent nicotinic and CNS signs	

Table 2: Toxic effects observed in two outbreaks of food-borne illness related to methomyl contamination^{32, 36}.

Clinical effects	Buchholz et al. * 2002	Tsai et al. ** 2003	
	No. Of subjects (%)	No. Of subjects (%)	Median latency to onset (min)
<i>Nausea</i>	102 (94%)	NR	NR
<i>General weakness</i>	8 (7%)	46 (84%)	5
<i>Dizziness</i>	76 (72%)	45 (82%)	5
<i>Vomiting</i>	54 (51%)	44 (80%)	5.5
<i>Sweating</i>	2 (2%)	41 (75%)	
<i>Lightheadedness / Floating sensation</i>	9 (8%)	39 (71%)	6
<i>Dyspnea</i>	NR	38 (69%)	5
<i>Headache</i>	56 (52%)	38 (69%)	6
<i>Blurred vision</i>	2 (2%)	37 (67%)	5
<i>Chills / Cold sensation</i>	51 (48%)	34 (62%)	
<i>Dysarthria</i>	NR	33 (60%)	5
<i>Diarrhea</i>	49 (46%)	32 (58%)	6
<i>Tremor of four limbs</i>	NR	31 (56%)	6
<i>Tingling / Paresthesia of limbs and trunk</i>	2 (2%)	28 (51%)	6
<i>Vertigo</i>	NR	27 (50%)	5
<i>Consciousness disturbance</i>	NR	24 (44%)	5
<i>Perioral numbness</i>	NR	23 (42%)	5
<i>Respiratory failure</i>	NR	22 (40%)	6.5
<i>Chest tightness</i>	NR	16 (29%)	6.5
<i>Face paresthesia</i>	NR	15 (27%)	5
<i>Bilateral calf cramping</i>	NR	14 (25%)	uncertain
<i>Fever</i>	15 (14%)	NR	NR
<i>Miosis</i>	NR	7 (13%)	uncertain
<i>Bradycardia</i>	NR	6 (11%)	179
<i>Incontinence</i>	NR	2 (4%)	uncertain
<i>Abdominal cramp</i>	62 (58%)	1 (2%)	120

NR = not reported

*outbreak of food-borne illness associated with methomyl-contaminated salt, N=107³²

**outbreak of food-borne illness due to methomyl contamination, N=55³⁶

3.2. Treatment

The basic principles of managing carbamate poisonings are based on: removal from the source of exposure, supportive measures, decontamination, seizure control, antidotes administration, and monitoring of acetylcholinesterase activity³⁵.

Mild intoxication

Patients suffering from a mild intoxication with a carbamate often do not need treatment. A patient who is either asymptomatic, or presents with mild clinical symptoms (i.e. normal vitals, pulse oximetry, and an acetylcholinesterase activity higher than 80% of lower reference value), and remains stable for 12 hours can be discharged²². Spontaneous recoveries of carbamate poisonings without medical treatment have occurred generally within 4 hours after exposure. In these cases symptoms were: headache, dizziness, weakness, excessive salivation, nausea, and vomiting²⁰.

Moderate and severe intoxications

In case of moderate to severe intoxications with a carbamate, respiratory management may be necessary because of respiratory muscle weakness and bronchorrhea. The use of activated charcoal is contraindicated because seizures or respiratory risk may develop rapidly. This could lead to a risk of aspiration. However, activated charcoal could be used in large ingestions when protective airway measures are available to apply³⁷. In practice, emesis is not induced because of the risk of aspiration. In severe intoxications where admission to the hospital is within 1 hour after ingestion, gastric lavage might be considered to empty the stomach.

Atropine is administered as an antidote in moderate and severe intoxications to treat muscarinic effects (e.g. salivation, lacrimation, defecation, urination, bronchorrhea, bradycardia)²². The dose and frequency of administration is based on the pulse rate of the patient. Following treatment with sufficient atropine, individuals have recovered from carbamate poisoning that produced such symptoms as: visual disturbances, profuse sweating, abdominal pain, incoordination, fasciculations, breathing difficulties, or changes in pulse rate²⁰. Seizures are treated with benzodiazepines. Cases of moderate or severe toxicity are admitted to intensive care with continuous monitoring, titration of antidotes, ventilation, and inotropes as needed²².

Oxime has occasionally been used for patients with severe toxicity to treat nicotinic effects (i.e., weakness, fasciculations, respiratory failure)²². Oximes are a group of drugs that have the capacity to reactivate inhibited acetylcholinesterase. One of the most common used oximes is pyridine-2-aldoxime methochloride, also known as 2-PAM or pralidoxime³⁵. However, the use of pralidoxime in carbamate intoxications is often not recommended, is considered unnecessary, or is specifically contraindicated. Cited reasons for hesitation to treat carbamate exposures with pralidoxime are:

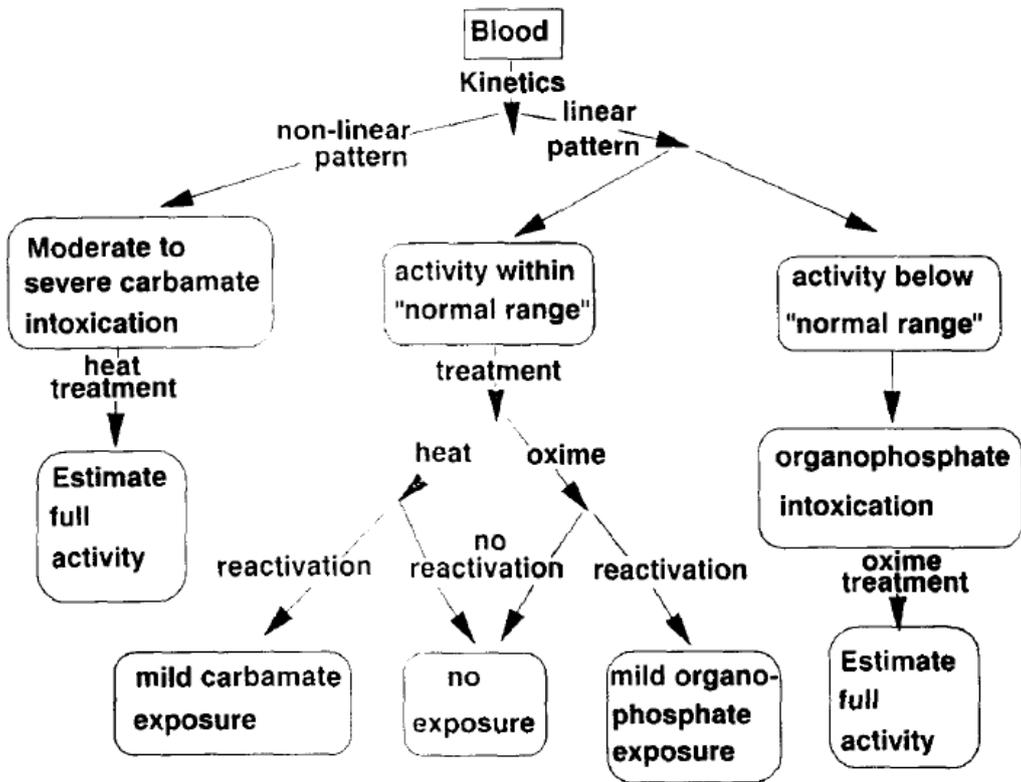
- autoreversibility of the carbamate AChE-bond (no “ageing” process as in organophosphates, paragraph 3.3),
- the short duration of binding between the carbamate moiety and AChE,
- based on accepted mechanisms of action, there is no rationale for pralidoxime to be effective, or
- pralidoxime may increase the toxicity of an exposure to carbamates².

On the other hand, when a poisoned patient presents with a cholinergic crisis and the poison is unknown, or an exposure to a mixture of cholinesterase-inhibitors (organophosphate and carbamate) has occurred, treatment with a combination of atropine and oximes is advised^{2, 35}. According to Rosman *et al.* (2009)³⁵, it is advised to use atropine in conjunction with oxime therapy even when carbamate intoxication is present with high certainty (with no information regarding the specific type of carbamate). Ekins and Geller (1994)² treated a methomyl poisoning with pralidoxime chloride that had a positive influence on the patient's clinical reversal. According to the textbook Medical Toxicology by Dart (2003)¹⁸, in many cases of carbamate exposure pralidoxime has been shown to be either beneficial, or at least not harmful.

3.3. Toxicity of carbamates relative to organophosphates

As mentioned earlier, anti-cholinesterase pesticides are subdivided into two main groups: organophosphates and carbamates. Organophosphates inhibit acetylcholinesterase in the same manner as carbamates do as described in the enzyme-substrate reaction (I) in paragraph 2.3. However, the reactivation rate is typically much faster for the carbamylated enzyme than for the phosphorylated enzyme. Furthermore, the phosphoryl residue may lose one of its alkyl groups by hydrolysis after which reactivation becomes virtually impossible. This process is called “ageing” of the phosphorylated enzyme and this does not occur with the carbamyl residue^{19, 38}. In other words, organophosphates produce a more permanent inhibition of the acetylcholinesterase enzyme whereas the carbamate-acetylcholinesterase bond is reversible producing a shorter intoxication and ultimately a relatively less threatening poisoning². Intoxications with organophosphates also lead to the cholinergic syndrome (Table 1). Symptoms of carbamate poisoning are indistinguishable from those of organophosphate poisoning, although they usually are of shorter duration³⁹. Unlike organophosphate, carbamate poisoning usually begins to resolve within several hours and disappears within 24 hours. Carbamates generally do not cross the blood-brain barrier as easily as organophosphates. Thus, in carbamate intoxications brain effects occur less frequently and generally with lower severity than with organophosphates. Unlike organophosphate intoxication, polyneuropathy, subacute neurotoxicity, delayed neuropathy, intermediate syndrome, or a deficiency in neurobehavioral testing, rarely appear in carbamate poisoning, although it has been documented in a few case reports³⁵. Intermediate syndrome is the delayed development of neuromuscular symptoms in patients recovering from a significant and often severe episode of acute organophosphate poisoning. The term intermediate is used because the syndrome occurs after the acute cholinergic crisis but before the development of delayed neuropathy. Clinical effects of intermediate syndrome typically begin 24 to 96 hours after exposure¹⁸.

The use of oximes for the treatment of intoxications with organophosphates is commonly accepted. As mentioned in the previous paragraph treating carbamate poisoning with oximes is controversial. It is valuable in cases of poisoning to differentiate between these two major classes of cholinesterase inhibitors³⁹. Rotenberg *et al.* (1995)³⁹ proposed an approach for the analysis of blood of an individual suspected to be poisoned by an unknown cholinesterase inhibitor, as described in Scheme 1. An Ellman assay (see next paragraph) was used for the real-time differentiation between carbamate and organophosphate inhibition of cholinesterase. In this setting, cholinesterase activity in blood needed to be measured continuously during incubation for about 1 hour, to be able to obtain the kinetic pattern of enzyme activity. The authors found that the enzymatic activity of carbamylated cholinesterase over time followed a non-linear kinetic pattern, whereas the activity of the phosphorylated enzyme was constant over time and did not show any recovery (Figure 4). The initial activity measured during the first 5 minutes of assay, represented the activity of the inhibited enzyme *in vivo*. *In vitro* reactivation of inhibited cholinesterase allowed the estimation of full potential activity of the enzyme prior to poisoning, so that the percentage of inhibition could be calculated. Reactivation of carbamylated cholinesterase was obtained by the incubation of diluted enzyme at 37°C (heat treatment) for 2.5 hours prior to the assay, whereas phosphorylated (non-aged) enzyme was reactivated by 30 minute incubation with oximes. In cases of mild exposure to cholinesterase inhibitors (<40% inhibition) the response of enzyme to *in vitro* reactivation served as a complementary test for exposure and for the nature of the inhibitor³⁹.



Scheme 1: Strategy for the differentiation between organophosphate and carbamate exposure in blood samples³⁹.

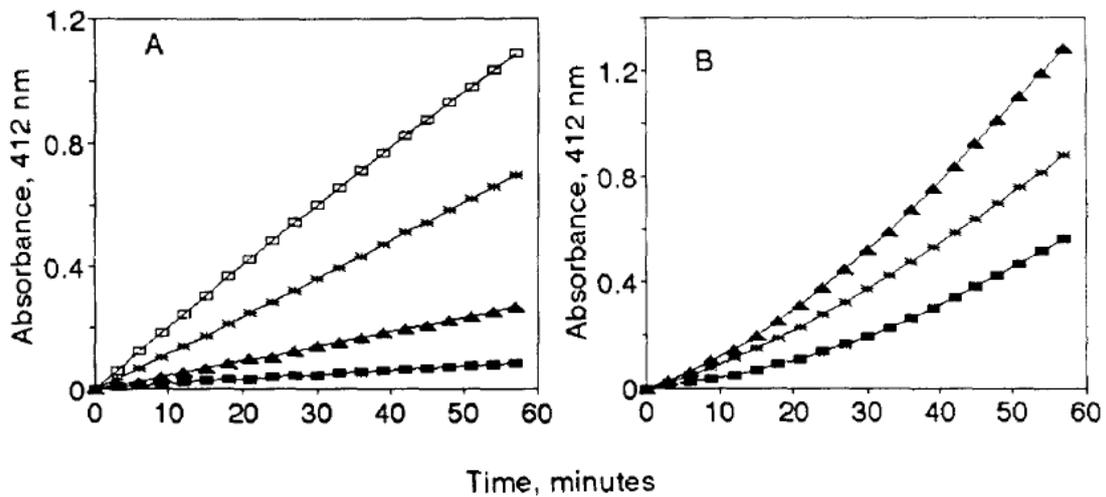


Figure 2: Kinetic profiles of plasma cholinesterase activity inhibited with different organophosphates (A) and carbamates (B). Panel A: ■, paraoxon 0.5 $\mu\text{mol/L}$; ▲, chlopyrifos 1 mmol/L ; ∗, O,O-dimethyl O-(2,2-dichlorovinyl) phosphate (DDVP) 25 $\mu\text{mol/L}$; □, malathion 1 mmol/L . Panel B: ■, pyridostigmine 10 $\mu\text{mol/L}$; ∗, carbaryl 0.5 mmol/L ; ▲, aldicarb 5 $\mu\text{mol/L}$ ³⁹.

3.4. Diagnostic tests

(Acetyl)cholinesterase activity

The inhibition of (acetyl)cholinesterase activity is used as a biomarker in poisonings with anti-cholinesterase pesticides. There are different types of cholinesterases in the human body which differ in their location in tissues, substrate affinity, and physiological function. The principal ones are acetylcholinesterase and plasma cholinesterases. Acetylcholinesterase is found in the nervous system and is also present in the outer membrane of erythrocytes. Plasma cholinesterases are a group of enzymes present in plasma, liver, cerebrospinal fluid, and glial cells. Under normal physiological conditions, acetylcholinesterase catalyzes the breakdown of acetylcholine that is the neurotransmitter responsible for propagation of nerve impulses at the site of cholinergic transmission (paragraph 2.3). However, its physiological role in blood is not understood. On the other hand, cholinesterase is a circulating plasma glycoprotein synthesized in the liver which probably hydrolyses naturally occurring esters. Both substrate and inhibitors react covalently with a serine residue in the esterases in essentially the same manner. This is because acetylation of the serine residue at acetylcholinesterase catalytic site is analogous to phosphorylation or carbamylation^{20, 40}.

The fraction of inhibited (acetyl)cholinesterase is often determined with the Ellman assay by Ellman *et al.* (1961)⁴¹. The method is based on the hydrolysis of the enzyme's substrate acetylthiocholine to acetic acid and thiocholine and the subsequent reduction by thiocholine of the 5,5'-dithiobis-2-nitrobenzoate ion to the yellow anion 5-thio-2-nitrobenzoate. The yellow color is measured in a spectrophotometer. To determine the inhibition of (acetyl)cholinesterase in an inhibition experiment the residual enzyme activity is expressed as a fraction of the activity of an unexposed sample, or a reference value^{19, 41}. Several assay kits are available commercially, all based on the same principle as the Ellman assay.

Johnson and Russel (1975)⁴² developed a radiometric assay for (acetyl)cholinesterase. In this assay, (³H-acetyl) choline is enzymatically hydrolyzed in a small reaction volume in a scintillation vial. The released [³H]acetate is then extracted into a toluene-based scintillator, added directly to the vial, without removing the reaction volume. The extracted [³H]acetate is then measured by counting the vial without further manipulations. The unhydrolyzed [³H]acetylcholine remains unextracted in the small aqueous reaction volume from which its weak β -particles of decay do not escape to excite the scintillator. However, in clinical settings this assay is seldom used mainly because of the radioactive waste that results from it.

The (acetyl)cholinesterase activities in blood can be determined in whole blood, erythrocytes, plasma, or serum. Activity measured in plasma is generally more sensitive, but activity in erythrocytes correlates somewhat better with clinical signs and symptoms. Depression of cholinesterase activity in excess of 50% of reference values is generally associated with cholinergic effects. In severe poisoning cholinesterase activity may be depressed by 90% of reference values. Correlation between cholinesterase levels and clinical effects in milder poisonings may be poor²². According to Martinez-Chuecos *et al.* (1990)¹⁵, determinations of plasma cholinesterase activity in methomyl poisonings could be useful even 20 hours after exposure. In those cases where plasma cholinesterase activity returned to normal values erythrocyte acetylcholinesterase measurements might still offer an alternative diagnostic method.

Methomyl concentrations in biological samples

Procedures used for analysis of methomyl in biological samples are high-performance liquid chromatography and gas chromatography, the latter with or without mass spectrometry⁴³. Methomyl is an unstable compound and it tends to dissociate to its oxime (Figure 5)⁴³ on gas chromatography column¹. Therefore, it is hard to study this pesticide with gas chromatography and to accurately determine the level of methomyl in human tissues for purposes of forensic toxicological examinations⁴³. Ito *et al.* (1998)⁴³ designed a more sensitive and reliable method to determine concentrations of methomyl in human whole blood. Methomyl was converted to its oxime and analyzed by gas chromatography-mass spectrometry with electron impact-selected ion monitoring as its oxime *t*-butyldimethylsilyl derivative.

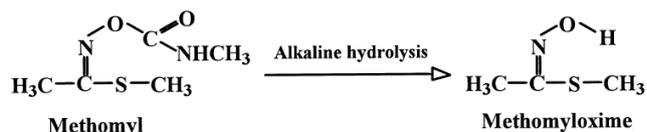


Figure 3: Structures of methomyl and methomyloxime⁴³.

3.5. Animal data

The activity of (acetyl)cholinesterase was found to be significantly decreased in several animal studies after administering methomyl. Al-Baggou and Mohammad (1999)³³ treated rats intraperitoneally with different doses (4 and 8 mg/kg) of methomyl. They found that methomyl at both doses significantly decreased erythrocyte acetylcholinesterase (40 and 43%) and plasma cholinesterase (23 and 31%) activities in comparison to the control values. Tsatsakis *et al.* (1998)⁴⁴ gave two rabbits subacute methomyl doses for 4 months. Each dose consisted of 30-40 mg methomyl dissolved in 1.5 L water and was consumed in 4 days. The total methomyl consumed by the rabbits during the 4 months was approximately 600 mg. Results from the treated rabbits were compared to those of two control rabbits. The reference value for acetylcholinesterase activity in blood for rabbits is 300-600 U/L. The values for the two control animals were 250-580 U/L and 270-600 U/L. Acetylcholinesterase activity in blood of the two treated rabbits was 240-390 U/L and 240-400 U/L. Tsatsakis *et al.* found that acetylcholinesterase measurements decreased during the dosing period, but were not accompanied by severe clinical signs. Mansour *et al.* (2009)²⁸ incubated rat erythrocytes during 4 hours at 37°C with different concentrations of methomyl; 0.0, 0.1, 0.5, 1.0, 1.5, and 2.0 mM. The erythrocyte sample showed a decrease in the activity of acetylcholinesterase. This decrease was concentration dependent. The acetylcholinesterase activity in the control was 83 U/mL. The remaining activity was 84%, 78%, 53% and 47% at the methomyl concentrations of 0.5, 1.0, 1.5, and 2.0 mM, respectively. Fayez and Bahig (1991)⁴⁵ determined acetylcholinesterase levels in rats following administration of oral daily doses of methomyl at 0, 4.8, and 8 mg/kg/day for 33 days. Acetylcholinesterase activity was determined in brain and blood after short periods of administration (15, 30, 45 minutes, 1, 2, 3, 4, 5, 6, and 7 hours). Maximum inhibition of blood acetylcholinesterase activity was shown after 30 and 15 minutes for the lower and higher dose, respectively. Maximum inhibition of brain acetylcholinesterase activity was shown after 45 and 15 minutes for the lower and higher dose, respectively. Fayez and Bahig stated that brain and blood acetylcholinesterase activity followed a similar pattern of inhibition, and restoration of activity in the first few hours. The recovery of the enzyme activity was attributed to the reversible reaction between pesticide and acetylcholinesterase. Padilla *et al.* (2007)²¹ dosed rats with methomyl (3 mg/kg in water) by gavage and each animal was killed at specific times after dosing. At a methomyl dose of 3 mg/kg, 67% inhibition in erythrocyte and 53% inhibition in brain acetylcholinesterase activity was observed 0.5 hours after dosing. By 4 hours after dosing, both the brain and erythrocyte acetylcholinesterase recovered to control levels. Excellent correlation was shown between erythrocyte and brain acetylcholinesterase inhibition if the radiometric assay was used. Fayez and Bahig (1991)⁴⁵ also studied the activity of the serum enzymes alanine transferase and alkaline phosphatase in rats orally dosed with methomyl at 0, 4.8, and 8 mg/kg/day for 33 days. Activity of both enzymes could be elevated with the early recognition of toxic hepatitis. A significant increase of the enzyme activity was observed after 4 days of treatment with the higher dose (8 mg/kg). However, the enzyme activity returned to normal values by the end of the intoxication period which suggested repair of the damaged liver cells. The effect on the kidney was studied by examining serum creatine levels and serum phosphate levels. Serum creatine level was significantly altered after 25 days with no dose relationship. Serum phosphate values were invariably elevated in the two dose levels, and a dose relationship did not seem to exist.

The study by Tsatsakis *et al.* (1998)⁴⁴ mentioned at the beginning of this paragraph also investigated the deposition of methomyl in rabbit hair. The total methomyl consumed by the rabbits during the 4 months was approximately 600 mg. Results from two treated rabbits were compared to those of two control rabbits. Methomyl concentration in whole hair measured in the control animals was 0 ng/mg. The main hair concentration in the treated animals was 1.3 and 1.4 ng/mg. No methomyl was detected in the distal section of the hair shaft. Methomyl was present in the second section, and the largest quantity was detected in the third section, the one nearest to the root. According to Tsatsakis *et al.*, the results suggested that methomyl was incorporated into the hair, but did not migrate along the hair shaft. Otherwise, the concentration of methomyl would have been constant in the 3 hair sections.

3.6. Human data (non-fatal cases)

Miyazaki *et al.* (1989)³⁴ reported a methomyl intoxication of a married couple. Both husband and wife had deliberately ingested methomyl. The man survived the ingestion, but the woman died. The methomyl concentration in the man was 0.01-0.1 µg/g in blood and 0.2 µg/g in urine. These samples were taken 28 hours after ingestion of an estimated amount of 30 mg/kg. According to the authors, the man had vomited more eliminating approximately more than half of the methomyl he had swallowed which attributed to his survival.

Ekins and Geller (1994)² described a poisoning due to an ingestion of an unknown amount of methomyl. On admission, erythrocyte acetylcholinesterase level was 8000 U/L (reference value 9000-18,000 U/L) and plasma cholinesterase level was 3000 U/L (reference value 7000-19,000 U/L). A total of 18 mg of atropine and 16 g of pralidoxime was given. After 48 hours, neurological status improved markedly. The patient remained in the hospital for nine days because of pneumonia.

Brahmi *et al.* (2005)⁴⁶ reported two cases of methomyl intoxication. A man was admitted to the hospital after ingestion of an unknown amount of methomyl. His acetylcholinesterase blood level was decreased at 1200 U/L (<20% of reference value >5000 U/L). A woman was admitted to the hospital half an hour after ingestion of 50 mL of a methomyl-containing product. Her blood acetylcholinesterase activity was 2500 U/L (<50% of reference value >5000 U/L). In both cases gastric lavage was performed along with atropine treatment. The acetylcholinesterase blood levels in both patients were normalized at day 3 of treatment. According to the authors, acute pancreatitis diagnosed in both patients was caused by the methomyl intoxication

Martinez-Chuecos *et al.* (1990)¹⁵ studied 11 cases of methomyl poisoning (Table3). The route of methomyl exposure was oral in 8 cases, and via skin in 3 cases. On admission, all patients presented with symptoms of poisoning. However, plasma cholinesterase activity was normal (3700-10,000 U/L) in 4 cases. The other 8 cases had plasma cholinesterase activities under the reference limits (ranging from 32 to 54%). The treatment applied included: gastric lavage, or washing the skin, administration of activated charcoal, and small doses of atropine based on the measured pulse frequency (average of total dose 4.3 mg). Plasma cholinesterase rose to 100% within 24 hours, except for 3 patients whose levels returned to normal by 48 hours. All the patients recovered within 24-48 hours, and no complications were detected in the acute stage or on further examination a month later.

Saiyed *et al.* (1992)⁴⁷ performed a health surveillance study in 22 healthy spraymen with a 5-day exposure to methomyl. The formulation for spraying consisted of 2.79-5.58 ml of 24% methomyl per liter water. Methomyl-related symptoms were observed in 5 (28%) spraymen: headache, nausea, excessive salivation, abdominal pain, and vomiting. ECG changes were found in 16 spraymen, and consisted of increased heart rate and T-wave changes. Plasma cholinesterase activity was measured with a Klett-Summerson colorimeter, and expressed in Klett units. Klett units are a measure of optical density of the sample. The more cholinesterase activity, the higher the number of Klett units. Average plasma cholinesterase activity in these 16 spraymen was 58.4 Klett units before exposure and 51.0 Klett units after exposure. The decrease in plasma cholinesterase activity was significant. No significant changes were noticed in erythrocyte acetylcholinesterase activities. Saiyed *et al.* concluded that the T-wave changes were the result of direct cardiac toxicity of methomyl because the inhibition of cholinesterase activity was minimal ($\pm 15\%$) and such changes were not commonly observed following severe exposure of other anti-cholinesterase compounds.

Table 3: Overview of clinical and analytical data on 11 methomyl poisonings¹⁵.

Case	Absorption route	Time-interval** (h)	Symptoms	Duration of symptoms (h)	Amount methomyl-containing product ingested (g)	Plasma cholinesterase activity* (U/L)		Total Atropine (mg)
						Admission	24h	
1	Digestive	2.5	D, M, S, ST, V	15	8	2000	3847	4.2
2	Digestive	14	AP, BV, D, M, S, V	18	12	8300	8250	3
3	Digestive	6	AP, AVB, BS, BV, M, T, V	46	10	1970	3000	7.8
4	Digestive	3.5	C, BS, L, M, S, T, V	32	2	1700	3920	0.8
5	Digestive	2	BS, Cr, M, Sa, ST, V	40	12	4300	5200	6
6	Digestive	2	M, T	20	16	2000	3915	5.4
7	Digestive	8	D, M	12	6	5320	5100	4.8
8	Digestive	3	M, S	10	14	1910	4000	1.4
9	Skin	4	AP, M, V	17	-	3950	4100	4.8
10	Skin	2	BS, M, T, V	40	-	1440	3590	5.4
11	Skin	4.5	AP, M, ST, V	28	-	1163	2265	3.6

AP= abdominal pain; AVB= auriculo-ventricular block; BS= increased bronchial secretion; BV= blurred vision; C= convulsions; Cr= cramps; D= diarrhea; L= lacrimation; M= miosis; S= stupor; Sa= salivation; ST= sinus tachycardia; T= tremor; V=vomiting

* normal value: 3700-10,000 U/L

**Period of time from intoxication to hospital admission

4. Post mortem findings

4.1. Diagnostic tests

For post mortem tissues and fluids, generally, the same parameters as in clinical samples (paragraph 3.4) are measured to obtain information on methomyl intoxications. These parameters are (acetyl)cholinesterase activity and methomyl concentration. Also, the same analytical techniques are used for these parameters. However, when the concentrations of methomyl in biological samples decrease due to post mortem reactions, the detection of this pesticide may become difficult⁴³. Methomyl is a sulfur containing compound and is instable in frozen post mortem biological samples. Biological specimens must therefore be treated with preservatives and be frozen to a temperature lower than -20°C if not immediately analyzed, in order to reduce losses of the analyte⁴⁸.

Hoizey *et al.* (2008)¹³ determined the anti-cholinesterase capacity of blood, urine and gastric content post mortem. This parameter would provide a rapid answer on the presence or absence of a cholinesterase inhibitor in biological samples. Hoizey *et al.* used a modification of a method by Mahieu *et al.* (1982)⁴⁹. In this method cholinesterase activity (P) was determined in all samples and in a blank control serum (T). Equal volumes of the blank control serum and of either blood, urine, or gastric content collected at autopsy were mixed and then incubated at 37°C for 15 minutes. Then, the cholinesterase activity was determined in each mixture (M). The anti-cholinesterase capacity of post mortem samples, expressed as a percentage, was then computed from the following equation:

$$100 \times \left[1 - \frac{2 \times (M)}{(T) + (P)} \right] \quad (\text{II})$$

As such, a value of 0% was indicative of the lack of cholinesterase-inhibiting compounds in the sample tested.

According to Prijono and Leighton (1991)⁵⁰, in birds the standard procedure for post mortem diagnosis of acute, lethal poisoning by anti-cholinesterase carbamate and organophosphate pesticides is the measurement of acetylcholinesterase activity in the brain. The authors looked for another biological molecule that was present in normal brain of birds at a constant ratio with acetylcholinesterase activity. This molecule should decompose after death at approximately the same rate as the disappearance of acetylcholinesterase activity. In this way it might be used to assess the approximate degree of actual acetylcholinesterase depression in cases of poisoning with anti-cholinesterase chemicals despite post mortem decomposition. Prijono and Leighton found the muscarinic acetylcholine-binding receptors to be useful for this purpose. These receptors were found to serve as a reasonable index of post mortem acetylcholinesterase activity. The muscarinic acetylcholine-binding receptors decomposed after death at a rate similar to acetylcholinesterase activity, and were not altered by acute lethal concentrations of an anti-cholinesterase pesticide⁵⁰.

4.2. Animal data

Ludke *et al.* (1975)⁵¹ reported that 20% inhibition of brain acetylcholinesterase in birds indicated exposure to anti-cholinesterase pesticides while inhibition of $\geq 50\%$ indicated death from poisoning. However, according to Prijono and Leighton (1991)⁵⁰, several factors may confound the interpretation of acetylcholinesterase activity in the brain in this diagnostic context, in particular, the wide variation in brain acetylcholinesterase activity among different species and the expected loss of acetylcholinesterase activity due to decomposition of tissue after death.

Tanaka *et al.* (1987)⁵² exposed rats to respirable methomyl powder for single and repeated periods: single 4 hour exposure, or during 3 months for 4 hours per day, or 5 days per week. After the rats were sacrificed blood was obtained and weights of lungs, spleen, kidneys and liver were measured immediately. Plasma cholinesterase activity was suppressed after exposure to methomyl powder. Marked suppression of plasma cholinesterase did not persist long after a single exposure. Even after repeated exposures for 3 months the degree of the suppression was not higher than that after a single 4 hour exposure. Erythrocyte acetylcholinesterase appeared slightly inhibited after acute methomyl powder exposure. No suppression of the enzyme activity was seen after excessive washing of the erythrocytes. Even after repeated exposures to methomyl powder acetylcholinesterase activity of the erythrocyte membrane did not differ significantly from that of the controls. According to Tanaka *et al.*, this indicated that methomyl which is bound to the erythrocyte acetylcholinesterase can be easily removed, and that even repeated exposure did not cause severe alteration of the activity of the enzyme. There was no significant histopathological abnormality between repeatedly exposed rats and controls. Lipid concentrations were measured in the lungs as indicator of respiratory response. There was no significant accumulation of lung lipids in this experiment.

El-Khawaga (2005)²⁶ administered a single dose of methomyl (7 mg/kg bw) to 6 mice by intraperitoneal injection. Methomyl was distributed in different organs, mostly in the lung (Table 4).

Table 4: Distribution of methomyl in different organs of in mice injected with methomyl in a dose of 7 mg/kg body weight²⁶.

Organ	Methomyl ($\mu\text{g/g}$ tissue)
Liver	37.69 \pm 3.4
Spleen	295 \pm 19.0
Kidney	225.7 \pm 34.0
Stomach	72.97 \pm 5.6
Intestine	142.2 \pm 17.0
Lung	803.3 \pm 54.0

Values expressed as mean \pm SD of six mice.

4.3. Human data (fatal cases)

Liddle *et al.* (1979)¹⁰ reported a fatal episode of accidental methomyl poisoning. Methomyl had been stored in an unlabeled tin can and was accidentally used in preparing a dish. Five men were transported to a hospital. Three of them were pronounced dead on arrival. The two persons who were still alive were treated with intravenous atropine and recovered. In the persons who died, several symptoms were seen immediately after eating. They jumped up, defecated, vomited, trembled, fell down, and complained of visual disturbances. Autopsy was performed 26 hours after death. Stomach contents had a strong smell of garlic. Stomach linings were highly congested as were lungs, tracheae and bronchi. The lethal dose of methomyl was estimated to have been between 12 and 15 mg/kg body weight.

As mentioned in paragraph 3.6, Miyazaki *et al.* (1989)³⁴ reported a methomyl intoxication of a married couple. The authors estimated that the woman had ingested the same amount as her husband (30 mg/kg). On admission, 1 hour after the ingestion, her methomyl blood concentration was 44 µg/g. She died 19 hours after ingestion, and at her autopsy 11 hours later her methomyl blood concentration was 0.2 µg/g.

In two studies by Tsatsakis *et al.* published in 1996, methomyl concentrations in organ tissues (liver, kidney, brain, thyroid) were found to be significantly lower than those in blood and vitreous humor. This fact was associated with the rapid biotransformation of methomyl in tissues. Analysis of stored tissue samples (frozen at -20°C) was performed between three and four weeks after death, while blood samples were analyzed immediately after autopsy to confirm cause of death^{1, 11}. In a later study of Tsatsakis (1998)⁴⁸ analytical determinations of methomyl in autopsy blood and organ tissues were performed immediately after death. Methomyl concentrations in various organ tissues were found to be similar or higher to those in blood (Table 5). Tsatsakis concluded that methomyl penetrates tissues of the reproductive system and other glands (thyroid, testes, ovaries, epididymis, submaxillary) Methomyl may accumulate in these tissues and glands in concentrations equal or higher than the methomyl concentrations in blood. The difference between the first two studies and the latter study was suggested to be caused by post mortem redistribution and biotransformation of methomyl⁴⁸.

Garcia-Repetto *et al.* (1994)⁵³ reported three lethal cases of methomyl poisoning. Methomyl blood concentration and methomyl stomach content were measured (Table 6). Methomyl concentration in bile was only reported in case 2.

In one of the Tsatsakis *et al.* studies published in 1996¹¹, four cases of lethal methomyl poisoning were reported. Methomyl concentrations in blood and the amount of methomyl in stomach content were measured in materials collected during autopsy (Table 7). Both stomach contents and blood had a garlic odour. Quantitative estimation of cholinesterase inhibition in blood plasma and serum showed high values of cholinesterase inhibition. The values corresponded to more than 90% inhibition of cholinesterase activity (180-300 U/L, reference values 3500-8500 U/L). In one of the cases samples of blood and urine were collected for analysis about 2 and 24 hours after methomyl ingestion. At that time the patient was still alive. However, brain death was diagnosed 10 days later. Two hours after ingestion methomyl concentrations in blood and urine were 28.0 mg/L and 33.0 mg/L, respectively. At that moment, cholinesterase activity was 210 U/L (6% of lower reference value, 3500-8500 U/L). Twenty-four hours after ingestion, methomyl concentration in blood was 0.2 mg/L. Concentration in urine was not determined at that time. Cholinesterase was re-activated to a level of 3345 U/L.

According to Tsatsakis *et al.*, these results show that cholinesterase activity recovers rapidly over the first 24 hours. No extra corporeal detoxification procedure like haemodialysis was used. According to the authors, the results were consistent with rapid elimination of methomyl.

Bertsias *et al.* (2004)⁴ reported six cases of methomyl intoxication. Initial methomyl blood levels and initial cholinesterase activities were registered (Table 8). In three cases the intoxicated person was found death. Methomyl blood concentrations ranged from 1.6 mg/L to 57.0 mg/L. Blood cholinesterase activity ranged between 5-11% of the lower reference value for blood cholinesterase activity (3500-8500 U/L).

Moriya and Hashimoto (2005)¹⁷ described a fatal poisoning of a man who had consumed tobacco leaves, methomyl, and tablets containing triazolam. Autopsy was performed 40 hours after his death. The lungs were heavily edematous. The stomach contained 170 g greenish liquid with a small amount of shredded tobacco leaves. Gastric mucosa was tainted green and congested with a large number of petechial hemorrhages. Analysis showed 21.8 mg nicotine, 304 mg methomyl and 1.69 mg triazolam, in the stomach (concentrations 128 µg/L, 1790 µg/L and 9.93 µg/L, respectively). Serum cholinesterase activities were 47-90 U/L/37°C (normal range for male: 200-440 U/L/37°C). Methomyl blood concentrations were 3-8 µg/L. Methomyl was also found in pericardial fluid (551 µg/L), brain (40-59 µg/kg), lungs (54 and 186 µg/kg), and femoral muscle (43 µg/kg). Although bile, myocardium, liver and kidney contained no methomyl, cerebrospinal fluid and vitreous humor contained substantial amounts of methomyl (2260 µg/L and 2680 µg/L, respectively). According to Moriya and Hashimoto, the findings strongly suggested that significant metabolism of methomyl occurred in the blood and tissues after death. Methomyl can be metabolized by carboxylesterases and cholinesterases in blood and tissues. Cerebrospinal fluid and vitreous humor have a low esterase activity which may be the reason for the high concentrations of methomyl found in these tissues. In this case, 11-45% of normal serum cholinesterase activity remained and miosis was not evident at death. The authors, therefore, concluded that the toxic effects of methomyl itself might have been mild to moderate. The nicotine concentrations in his blood might have been sufficient to potentiate the toxicity of methomyl and cause respiratory paralysis. Therefore, death was concluded to be caused by an additive interaction between methomyl and nicotine.

Hoizey *et al.* (2008)¹³ examined a case of thiodicarb poisoning. A woman was found dead in her car, surrounded by empty packages of medicines and a bottle of Larvin, a pesticide containing thiodicarb. An autopsy was performed 3 days after death. All post mortem samples were stored at -20°C. Specific analysis of thiodicarb and methomyl was performed on all fluids and tissues collected during autopsy. The capacity to inhibit cholinesterase (paragraph 4.1) was determined in blood, urine and gastric content collected at autopsy and was 83%, 82%, and 32%, respectively (normal value: 0%).

Although thiodicarb was only detected in gastric content (24.3 mg/L), its methomyl metabolite could be quantified in most postmortem tissues and fluids: gastric content (19.9 mg/L), peripheral blood (0.7 mg/L), urine (8.5 mg/L), bile (2.7 mg/L), liver (0.7 mg/kg), kidney (1.7 mg/kg), lung (1.5 mg/kg), brain (9.3 mg/kg), and heart (3.6 mg/kg). Concentrations of methomyl in most tissues and fluids, particularly brain and heart, were higher than in peripheral blood. In contrast to the case reported by Moriya and Hashimoto (2005)¹⁷, methomyl was not detected in vitreous humor although this was described as a specimen of choice for detecting carbamates post mortem¹⁷. Hoizey *et al.* showed that after oral ingestion thiodicarb was not detected in any of the tissues of fluids analyzed. This confirmed the important distribution of methomyl throughout the body.

Tsatsakis *et al.* (2001)²⁵ reported an acute fatal poisoning by methomyl caused by inhalation and transdermal absorption. The patient was a farmer who owned a greenhouse where he worked with pesticides without any precaution. During the last week he had symptoms of hypotonia and bradycardia. His practitioner attributed these symptoms to anxiety and prescribed bromazepam. The man was found comatose in his greenhouse and was admitted to the hospital 3.5 hours later. Cholinesterase activity and blood methomyl concentration were measured from admission until death (Table 9). The treatment included: gastric lavage and activated charcoal, skin washing, and atropine administration. Haemoperfusion and oxime treatment were not applied. Cholinesterase activity recovered (reference values 3500-8500 U/L), and blood methomyl concentration decreased during the days measured. However, his general condition gradually deteriorated leading to systemic inflammatory response syndrome and death three days after his admission, because of multiple organ failure syndrome. At autopsy, congestion of viscera and pulmonary edema were found. Excessive degenerative phenomena were detected in the liver, spleen, pancreas, adrenal glands, and urinary tract. Methomyl was detected in liver tissue but not in heart blood or gastric contents. The pesticide deposition in hair was also measured and was found to be 4 ng/mg in the proximal section of the hair.

Ito *et al.* (1998)⁴³ examined solid tissues from an autopsy performed 2 years prior to their investigation. The tissue was from a man who was found dead at a farm where he worked. Methomyl was quantitatively present in his gastric content. Tissues were preserved in formaldehyde solution at room temperature for 2 years. Methomyl was detected in every sample examined. Concentrations of methomyl found were heart (25.3 mg/kg), brain (22.1 mg/kg), kidney (8.63 mg/kg), spleen (5.66 mg/kg), and lung (14.9 mg/kg). In a preliminary study, Ito *et al.* showed that the methomyl concentration in whole blood kept at 25°C decreased rapidly to about 1/30 after one week and 1/100 after two weeks. Therefore, the authors concluded that methomyl concentrations of tissues in this case were supposedly high at time of death, and drastically decreased to trace level during storage of the samples.

Table 5: Range / mean methomyl concentration (mg/L) in biological fluids and tissues from 3 studies^{1, 11, 48}.

Autopsy Material	Tsatsakis <i>et al.</i> 1996a ¹¹ +b ¹ †	Tsatsakis 1998 ⁴⁸ ‡
<i>Blood</i>	5.6-63.5	19.8
<i>Liver</i>	0.1-1.2	9.0
<i>Kidney</i>	0.2-2.8	18.1
<i>Brain</i>	0.07-0.31	2.1
<i>Vitrous humor</i>	1.4, 7.0**	NR
<i>Thyroid</i>	0.05*	20.3
<i>Testis</i>	NR	11.9***
<i>Epididymis</i>	NR	12.2***
<i>Ovaries</i>	NR	31.4*
<i>Adrenal gland</i>	NR	16.6
<i>Submaxillary gland</i>	NR	18.5

NR= not reported

†Range of methomyl concentrations (mg/L) from 8 autopsies^{1, 11}.

‡Mean methomyl concentrations (mg/L) from 4 autopsies⁴⁸.

*Based on 1 autopsy

**Based on 2 autopsies

***Based on 3 autopsies

Table 6: Methomyl concentrations in blood, stomach content and bile in three lethal cases⁵³.

Case	Methomyl concentration		
	Blood (mg/L)	Stomach content (g/L)	Bile (mg/L)
1	33.9	17.8	NR
2	10.7	11.6	1.4
3	8.0	5.3	NR

NR= not reported

Table 7: Methomyl blood concentrations and methomyl contents in stomach found at autopsy in 4 cases¹¹.

Case	Interval death-autopsy (h)	Methomyl concentration blood (mg/L)	Methomyl in stomach (g)
1	12	33.0*	2.7
2	5	54.5*	2.1
3	8	8.4*	1.8
4	5	5.6**	1.5

*Average of concentration determined with HPLC and concentration determined with GC/MS

** Concentration determined with HPLC

Table 8: Initial methomyl blood concentration and cholinesterase activity in 6 cases of methomyl intoxication⁴.

Case	Methomyl blood concentration (mg/L)	Cholinesterase activity (U/L)
1	1.6	380
2 #	5.6	180
3 #	8	199
4	28	245
5 #	35	262
6	57	300

person was found death

Table 9: Inhibition of cholinesteras activity (from the lowest reference value = 3500 U/L) and blood methomyl concentration²⁵.

	On admission	Day 1	Day 2	Day 3
Cholinesterase activity (U/L)	380	860	2400	3200
Percentage of inhibition	89%	75%	31%	9%
Blood methomyl concentration (mg/L)	1.6	0.8	0.6	0.1

5. Discussion

Interpretation of the information given in this review is difficult due to a number of reasons. First, the number of articles published on methomyl intoxication is limited. Secondly, methomyl is an instable compound, even in frozen post mortem biological samples. Thirdly, the cases described often lack information. For instance, on the delay between methomyl exposure and diagnostic testing, or the amount of methomyl the person was exposed to. Fourthly, it is often hard to distinguish aspects of methomyl intoxications from aspects of intoxications with other carbamates, or even organophosphates.

(Acetyl)cholinesterase activity / capacity

As mentioned in paragraph 3.4, the (acetyl)cholinesterase activities in blood can be determined in whole blood, erythrocytes, plasma, and serum. The type of cholinesterases present and reference values for (acetyl)cholinesterase activity differ per type of sample. Due to these differences comparison of (acetyl)cholinesterase activity levels found in the described cases is problematic. (Acetyl)cholinesterase activities in whole blood are most useful to compare with findings obtained at the Dutch Forensic Institute since this is the main matrix available for forensic investigation.

Fayez and Bahing (1991)⁴⁵ stated that in the first hours after exposure brain and blood acetylcholinesterase activity in rats followed a similar pattern of inhibition and recovery. This finding might be useful in forensic investigation post mortem. However, the study of Fayez and Bahing was based on rats. Reference values for acetylcholinesterase activity in human brains are not available. Accordingly, this correlation between blood and brain acetylcholinesterase is not relevant for humans unless further research on this matter is performed.

According to Brahmī *et al.* (2005)⁴⁶, the reference value for blood acetylcholinesterase activity in humans is >5000 U/L. Two cases of methomyl intoxication were reported. Acetylcholinesterase activities of 1200 U/L (<20%) and 2500 U/L (<50%) were found. Both patients recovered from the intoxication. This is consistent with data from the Poisindex® (2010)²². Both intoxications had acetylcholinesterase activity levels <50% of reference values, so cholinergic effects were expected. However, the acetylcholinesterase levels were not depressed by 90% or more compared to the reference values that can be found in severe cases.

Hoizey *et al.* (2008)¹³ studied a case of thiodicarb poisoning, in which a woman was found death in her car. The capacity to inhibit cholinesterase was found to be 83% in blood (reference value is 0%). This finding suggests that an anti-cholinesterase capacity of 83% is related to death. However, information on the interval between death and measuring the capacity is lacking. Perhaps the capacity had a higher or lower value at time of death. Also, this was the only article found in literature that used anti-cholinesterase capacity in methomyl intoxications.

Diagnostic tests

Padilla *et al.* (2007)²¹ found an excellent correlation between erythrocyte and brain acetylcholinesterase inhibition in rats but only if a radiometric assay was used. The correlation became non-existent with the spectrophotometric assay based on the Ellman method⁴¹. According to Padilla *et al.*, the Ellman assay with traditional unmodified conditions is likely to underestimate the acetylcholinesterase inhibition in tissues from carbamate-treated animals. According to the authors, more concentrated tissue samples, carrying out the Ellman assay more rapidly to limit the affect of reactivation, and performing the assay at a lower temperature might make the results more comparable to the radiometric assay. Bosgra *et al.* (2009)¹⁹ also stated that the acetylcholinesterase activity measured by the Ellman assay underestimates the inhibition before the assay.

According to the authors, this underestimation was due to relatively high reactivation rates of carbamates. Bosgra *et al.* state that measuring acetylcholinesterase after adjusting the Ellman protocol or by using a radiometric assay would still be influenced by the high reactivation of acetylcholinesterase. Therefore, Bosgra *et al.* suggested that measured acetylcholinesterase activities should be corrected for this reactivation. This correction is only applicable to the calculation of the maximal inhibition on an earlier point in time. It is not needed in practice since treatment is based on the clinical presentations rather than on the inhibition of acetylcholinesterase activity.

As mentioned in paragraph 3.4, methomyl is an unstable compound and it tends to dissociate on gas chromatography column to its oxime¹ (Figure 5)⁴³. Therefore, it is not advisable to measure this pesticide with gas chromatography in human tissues for purposes of forensic toxicological examinations⁴³. Ito *et al.* (1998)⁴³ stated to have designed a more sensitive and reliable method to determine concentrations of methomyl in human whole blood. Herein, methomyl was converted to its oxime and analyzed by gas chromatography-mass spectrometry with electron impact-selected ion monitoring as its oxime *t*-butyldimethylsilyl derivative. However, at the time of writing this review, this method was not referred to in other relevant articles. Therefore, it is unknown if this method is a good alternative.

Prijono and Leighton (1991)⁵⁰ found that in quail brains, muscarinic acetylcholine-binding receptors might be used to assess the approximate degree of actual acetylcholinesterase depression in cases of anti-cholinesterase intoxication despite post mortem decomposition. Further research would be necessary to determine if these muscarinic acetylcholine-binding receptors might be used in human brains or even other human tissues as well. According to Ludke *et al.* (1975)⁵¹, not all cholinesterase inhibitors readily penetrate the blood-brain barrier. Therefore, mortality might occur without an accompanying decrease in brain acetylcholinesterase activity. As mentioned earlier in this chapter, Fayez and Bahing (1991)⁴⁵ stated that in the first hours after exposure, the acetylcholinesterase activity in rat brains followed a comparable pattern of inhibition and restoration as the acetylcholinesterase activity in rat blood. This indicates that methomyl is capable of penetrating the blood-brain barrier. The studies from Prijono and Leighton (1991)⁵⁰ and Fayez and Bahing (1991)⁴⁵ were both performed in non-human species. In studies by Tsatsakis *et al.* (1996)^{1, 11}, Ito *et al.* (1998)⁴³, Moriya and Hashimoto (2005)¹⁷, and Hoizey *et al.* (2008)¹³, methomyl was found in the human brain as well.

On the whole, in humans it is probably not feasible to use muscarinic acetylcholine-binding receptors to assess the acetylcholinesterase depression in practice.

Clinical

To be able to predict the clinical outcome of a methomyl intoxication a variety of data is required. The most important ones are: the amount of methomyl a person was exposed to, weight of the intoxicated person, methomyl concentration in blood, and the interval between exposure and measurement of the parameter. The extent of (acetyl)cholinesterase inhibition might give an indication of the severity of the methomyl intoxication. Most of the studies mentioned here lack at least one of these data. Additionally, the reference range of human cholinesterase and acetylcholinesterase activity varies extensively in the population, and depends on a variety of factors, among which are age, sex and hormonal condition³⁹. But the reference range also depends on the conditions used in the different assay kits. According to Jensen *et al.* (1995)⁵⁴, the plasma cholinesterase activity in children aged 10 years or less did not vary significantly with age or sex. However, the authors found that from the age of about 10 years the enzyme activity decreased statistically significant in both males and females. At about 60 years of age the enzyme activity returned to the level seen before puberty. Patients can experience clinically significant anti-cholinesterase poisoning and still have a erythrocyte cholinesterase level that is within the low reference range.

Erythrocyte cholinesterase activity may also be affected by genetic influences and certain medical conditions, such as anemia or haemolysis, and by ongoing therapy with drugs such as anti-malarials or oral contraceptives. Cholinesterase of plasma and serum is replaced less rapidly in individuals who have liver damage. The plasma and serum cholinesterase activity may be depressed in those individuals having cirrhosis, chronic toxic, or viral hepatitis, malignancies, hepatic or obstructive jaundice, decompensated heart disease, allergic disease, and pregnancy⁵⁵.

In the articles discussed in this review, different reference values of enzyme activities are used depending on measurement techniques and type of blood samples used by the authors. Comparison of the studies on methomyl intoxication is, therefore, difficult to do.

The study by Martinez-Chuecos *et al.* (1990)¹⁵ in paragraph 3.6 and Table 3, seemed to have most of the required data available. From the data of this study, the average clinical and analytical data for a methomyl poisoning by ingestion can be calculated. All cases of oral exposure were adults (29-60 years) except for case 4 (10 years). On average, the adult patients had ingested 11 g (6-16 g) of methomyl-containing product. They were admitted to the hospital on average 5.5 hours (2-14 h) after ingestion. Their plasma cholinesterase activity on admission was on average 3685 U/L (1910-8300 U/L), and 4758 U/L (3000-8250 U/L) after 24 hours. Compared to the reference values used in this study (3700-10,000 U/L) the average plasma cholinesterase activity on admission was even close to normal. The average amount of atropine used was 4.7 mg (1.4-7.8 mg). Weights of the patients were not given. According to the International Program on Chemical Safety⁵⁶, in risk assessment an average weight of 60 kg needs to be used for adults. The average dose ingested by the adult patients would be 0.18 g/kg or 180 mg/kg. This confirms that the amount ingested is based on the entire product containing methomyl and not on the amount of methomyl actually ingested. Otherwise the lethal dose of methomyl 12-15mg/kg estimated by Liddle *et al.*¹⁰ would be exceeded. The amount of actual methomyl ingested is unknown. Therefore it is difficult to draw conclusions on the amount methomyl that would lead to the cholinesterase activity and the accompanied clinical outcome found in this study.

In two cases reported by Tsatsakis *et al.* in (1996)¹¹ and (2001)²⁵ the patients died although their cholinesterase activities returned to normal and their methomyl blood concentration decreased during treatment. The fatal outcome is probably due to slow reabsorption of methomyl from the tissues back into the blood. Therefore blood levels may remain toxic and prevent recovery of the patients. In both cases no extra corporeal detoxification procedures like haemoperfusion or haemodialysis were performed that might have enhanced the patients' survival.

Treatment of carbamate intoxications with oximes is controversial as mentioned in paragraph 3.2. However, when in a methomyl intoxication oxime administration is considered, blood specimens need to be drawn in advance^{2, 11}. This way, the regeneration of (acetyl)cholinesterase by the oxime will not influence the initial (acetyl)cholinesterase activity. The initial (acetyl)cholinesterase level might be used to estimate the extent of exposure to methomyl and the severity of the intoxication.

Post mortem

Post mortem methomyl concentrations in humans have been found in several tissues: heart, liver, kidney, spleen, lung, brain, vitreous humor, femoral muscle, hair, thyroid, adrenal gland, submaxillary gland, testis, epididymis, and ovaries^{1, 11, 13, 17, 25, 43, 48}. Methomyl was also detected in fluids: blood, bile, stomach content, pericardial fluid, cerebrospinal fluid, and urine^{1, 11, 13, 17, 53}. Other observations in post mortem tissue included: congestion of gastric mucosa with or without petechial hemorrhages; congestion of viscera among which, lung tracheae, and bronchi^{10, 17, 25}. Also edematous lungs were found at autopsy^{17, 25} as well as degenerative phenomena in liver, spleen, pancreas, adrenal glands, and urinary tract²⁵.

Methomyl concentrations decrease in biological samples due to post mortem reactions⁴³. According to Tsatsakis *et al.* (1998)⁴⁸, immediately after death methomyl penetrates tissues of the reproductive system and glands in concentrations similar to, or higher than in blood,. In two earlier studies (1996)^{1, 11}, the same authors found significant lower methomyl concentrations in several organ tissues, compared to methomyl concentrations found in blood and vitreous humor. Here, analysis of the tissues was performed 3 to 4 weeks after death. According to Moriya and Hashimoto (2005)¹⁷, methomyl was found in cerebrospinal fluid and vitreous humor 40 hours after death in higher concentrations than was found in blood. These studies indicated that there is significant metabolism of methomyl in blood and tissue, after death. Cerebrospinal fluid and vitreous humor contained higher methomyl concentrations due to their low esterase activity¹⁷. Hoizey *et al.* (2008)¹³ reported a thiodicarb intoxication. Autopsy was performed 3 hours after death and thiodicarb was only recovered from the gastric content. Methomyl, however, was found in several tissues. According to Hoizey *et al.*, these findings were indicative of an extensive ante mortem distribution of methomyl, and in agreement with the high lipid solubility of methomyl. However, methomyl is lipophobic, therefore, it does not uphold this explanation. This study, did show that methomyl traces found in biological samples might also indicate a thiodicarb intoxication.

Ito *et al.* (1998)⁴³ examined 2-year-old biological samples of a fatal case of methomyl intoxication. High methomyl concentrations were found in the tissues (paragraph 4.3). The authors presumed that these concentrations were just a fraction of the methomyl concentration at the time of death. The methomyl concentrations found in the 2-year-old biological samples were generally higher than the concentrations found in other studies where tissues were analyzed within maximal 4 weeks after death^{1, 11, 13, 17, 48}. The tissues examined by Ito *et al.* were preserved in formaldehyde. Formaldehyde inhibits enzyme activity. According to Couteaux (1955)⁵⁷, cholinesterase offers unequal resistance to the inactivating effect of formaldehyde. In this case the formaldehyde may have prevented the methomyl to degrade in the tissues during the two years of preservation. Therefore, the methomyl concentration found in the preserved tissue cannot simply be attributed to be a small fraction of the methomyl concentration at the time of death.

Metabolites can be used to determine if the original compound was present in the human body. They might also give an indication of the size of the dose of the original compound, and the time past since the exposure. However, the metabolism of methomyl in humans is largely unknown¹¹. The two main metabolites of methomyl in animals, acetamide and acetonitrile, have small residue levels. If the same is true for humans, they are probably not adequate as indices for methomyl intoxication. Further research might reveal if other, more suitable metabolites are present in humans to determine if methomyl intoxication took place.

Methomyl intoxication in rats and mice has led to oxidative stress due to altered levels and activities of antioxidant defense system enzymes and lipid peroxidation²⁶⁻²⁸. Further research might elucidate whether these altered levels and activities can also be found in humans. If that is the case, the antioxidant defense system enzymes and lipid peroxidation might be used as indices of methomyl intoxications.

Hair has been used as biomarker for exposure to drugs of abuse, medicines, and heavy metals. Therefore, it might also be a biomarker for chronic (internal), and recent (external) exposure to methomyl as well. The main advantage of hair is that it retains trapped information for prolonged periods of time. This is attributed to the absorption and trapping mechanism that exists in the hair, taking place during keratinisation of the newly formed cells. Hair consists of long shafts created of closely packed cells that emerge from the follicles. Hair is rich in keratins, a family of proteins with a high content of sulfur. Inside the shaft, keratin forms long fibers linked to each other by sulfur bridges and other types of bonds between keratin and other proteins creating a very stable structure. The hair could be described as cross-linked polymer containing a large number of chemical functional groups capable of trapping small molecules. Human hair consists of 65-95% proteins, 15-35% water and 1-9% lipids.

Substances in the blood-circulating system which enter the hair via the follicle are trapped and retained in specific parts of the hair. Water-soluble compounds excreted into sweat and sebum from the skin may also be incorporated in the hair⁴⁴.

Tsatsakis *et al.* (2009)⁵⁸ conducted an epidemiological study in Crete. Experimental data from this study confirmed the presence of organophosphate pesticides in human hair samples. In 1998, Tsatsakis *et al.*⁴⁴ found that methomyl was incorporated in rabbit hair after they were exposed to this pesticide. Methomyl did not migrate along the hair shaft. In 2001, Tsatsakis *et al.*²⁵ found methomyl in the proximal section of hair, at the autopsy of a farmer, who had worked with pesticides for years, without any precaution. This might indicate that the farmer had been exposed chronically to methomyl, in a period just before his death. Therefore, hairs might be used to detect if chronic exposure to methomyl took place, preceding death. Before analyzing, hair should be washed properly to be able to distinguish between internal, and external exposure to methomyl. Previous studies have indicated that hair color is a crucial parameter that determines the amount of chemicals that may bind to hair, especially if absorption of the substance in hair occurs through the bloodstream, during the keratinisation step of the hair shaft. More specifically, it has been found that the concentration of many substances measured in hair is proportional to the melanin content of the hair shaft. Therefore, it is important to account for differences in methomyl concentrations in hair, due to the color of the hair that is analyzed. All in all, hair analysis gives valuable information about exposure to pesticides. However, up till now, evidence on the dose-response relationship between chemical ingestion and chemical concentrations in the hair is contradicting⁵⁸.

Contradictory results are found in the study by Bertias *et al.* (2004)⁴. The authors reported on 6 lethal cases of methomyl intoxication (paragraph 4.3). The results (Table 8) seem to give a linear relationship between initial methomyl blood levels and initial blood cholinesterase activities. The cholinesterase activity in blood tended to be higher at higher concentrations of methomyl in the blood except for case 1. It would be expected that cholinesterase activity is inhibited more at larger methomyl exposures. However, in 3 out of 6 cases the person was found dead. In all 6 cases the extent of the interval between death and the analysis of the blood was unknown. Post mortem metabolism of methomyl in blood and tissues have probably altered the methomyl concentration in blood, and/or the cholinesterase activity in blood.

Tsatsakis *et al.* (1996)¹¹ reported on 4 lethal cases of methomyl intoxication, paragraph 4.3. For each case, the amount of methomyl in the stomach, the methomyl concentration in blood, and the interval between death and autopsy were given (Table 7). The methomyl concentration in blood tended to be higher at higher amounts of methomyl in the stomach, taking into account the different intervals between death and autopsy. It would be informative to compare these data with findings on cholinesterase activities in the blood, to be able to say something more on the relationship between amount of methomyl ingested, methomyl concentration in blood, and blood cholinesterase activities. The authors, however, only mentioned that cholinesterase activities ranged between 180-300 U/L (reference values 3500-8500 U/L), without specifying the cholinesterase activities per case.

6. Conclusion

Some important clinical and post mortem aspects may be considered in forensic investigations of suspected methomyl intoxications.

Methomyl is highly toxic, especially after acute exposure. It can exert its toxicity via different routes of exposure. Carbamates like methomyl inhibit (acetyl)cholinesterase. This leads to symptoms that are part of the cholinergic syndrome. In carbamate pesticide intoxications, (acetyl)cholinesterase activities of <50% of baseline are expected to lead to cholinergic effects. Severe intoxications often have a depression of (acetyl)cholinesterase activities of 90% or more.

The inhibition of (acetyl)cholinesterase activity is used as a biomarker in poisonings with methomyl. In most cases this activity is measured with an Ellman assay. The extent of (acetyl)cholinesterase activity inhibition might give an indication of the severity of the methomyl intoxication. Therefore, the reference values of (acetyl)cholinesterase activity needs to be determined keeping in mind possible deviations due to age, medical background, and/or applied treatment, but also the conditions of the used assay kit. The inhibition of acetylcholinesterase by carbamates is reversible and the inhibited enzyme can be reactivated rapidly. Therefore, other biomarkers might be more reliable in suspected methomyl intoxications.

The methomyl compound can be analyzed in biological samples. Procedures used for analysis are high-performance liquid chromatography and gas chromatography, the latter with or without mass spectrometry. Methomyl concentrations in cerebrospinal fluid and vitreous humor may be higher for a longer period of time due to their low esterase activity. Hairs might be used to detect past exposure to methomyl. Additionally, it is important to keep in mind that intoxications with the carbamate thiodicarb also lead to traces of methomyl in human tissues and fluids, and possibly hair.

Methomyl is rapidly metabolized because of its easy degradation and lack of chemical stability. Its elimination is also fast. Therefore, the detection of this pesticide may become difficult. Methomyl is even instable in frozen post mortem biological samples. Biological specimens must, therefore, be treated with preservatives and be frozen to a temperature lower than -20°C if not immediately analyzed, in order to reduce losses of the analyte.

Alternative measures might give a better indication of a methomyl intoxication. Metabolites may give an indication of the dose of the original compound, and the time past since the exposure. However, the metabolism of methomyl in humans is largely unknown. Altered levels and activities of antioxidant defense system enzymes and lipid peroxidation might be useful as indices of oxidative stress due methomyl intoxication.

The mechanism of toxicity of carbamates is similar to that of organophosphates. They both result in the cholinergic syndrome and therefore, the symptoms of organophosphate and carbamate intoxications are indistinguishable. However, the recovery of the acetylcholinesterase enzyme activity can be used to differentiate between carbamates and organophosphates. The enzymatic activity of carbamylated cholinesterase over time follows a non-linear kinetic pattern, whereas the activity of the phosphorylated enzyme is constant over time and shows no recovery.

The use of oximes for the treatment of intoxications with organophosphates is commonly accepted, while treating carbamate poisoning with oximes is controversial. However, when oxime administration is considered in a methomyl intoxication, blood specimens need to be drawn in advance. This way, the regeneration of (acetyl)cholinesterase by the oxime will not influence the initial (acetyl)cholinesterase activity.

The number of articles published on methomyl intoxications is limited. Several aspects of these intoxications require further research. Animal studies have shown interesting alternative diagnostic parameters. Further research might reveal if these parameters can be used in humans as well. Especially, the clarification of methomyl metabolism in humans is needed. Also, possible relationships between: amount of methomyl ingested, methomyl concentration in blood, and blood (acetyl)cholinesterase activities require further research. Furthermore, evidence on the dose-response relationship between chemical ingestion and chemical concentrations in the hair is contradicting and needs further investigation.

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