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Analysis of Methaqualone in Autopsy Material

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Summary. A method for detection and determination of methaqualone and some of its metabolites in autopsy material is described, involving extraction, ultra-violet spectrophotometry, gas and thin-layer chromatography.

Twenty autopsy cases of intoxications were investigated in a detailed fashion. In most cases, the concentration of metabolites in blood, liver and kidney were considerably lower than those of the parent drug, especially when other drugs and/or ethanol also were found. Only small amounts of methaqualone and "free" metabolites were present in the urine. However, after acid hydrolysis, considerable quantities of metabolites were liberated.

A statistical survey of autopsy cases where methaqualone was detected in routine analyses are given. Between, 1964 and June, 1970, methaqualone was detected in 165 autopsy cases, about 90% of these being suicides. 22 cases were intoxications with methaqualone alone. In the remaining ones, death was due to combinations of drugs (including ethanol), or due to physical factors.

From the material presented here, the lower limit of "lethal concentration" in blood seems to be 1—2 mg of drug per 100 ml in pure methaqualone intoxications.

Key-Words: Methaqualone poisoning—Methaqualone analysis—Methaqualone in post-mortem material.

Zusammenfassung. Eine Methode zur Isolierung und Identifizierung von Methaqualon (2-Methyl-3-*o*-4(3H)-chinazolinon) in Leichenmaterial wird beschrieben. Proben von Leber bzw. Niere werden mit Äthanol homogenisiert und die Suspension zentrifugiert. Die klare Lösung wird abdekantiert und eingedunstet. Im übrigen, sowie auch für Blut und Harn, wurde das Verfahren von Bonnichsen u. Mitarb. (1961) und Maehly und Bonnichsen (1966) verwendet.

Um unverändertes Methaqualon in den Extrakten zu bestimmen, wurden diese gaschromatographisch untersucht. Als stationäre Phase wurde 1% HiEff 3A verwendet (ein Polyester von Applied Science Laboratories, U.S.A.) und als Träger Gas Chrom Q bei einer Kolonnentemperatur von 190°. Unter diesen Verhältnissen wurden Metaboliten des Methaqualons nicht eluiert.

Mit Hilfe der Ultraviolett-Spektrophotometrie wurde die Gesamtmenge von Methaqualon und gewissen Metaboliten mit annähernd gleichen Spektren bestimmt. In einigen Fällen wurden Extrakte von Blut, Leber und Niere auch dünnschicht-chromatographisch untersucht. Auf den Chromatogrammen konnten außer Methaqualon 3 andere Flecken (manchmal nur 1 oder 2), die mit Dragendorffs Reagens angefärbt werden und Metaboliten von Methaqualon darstellen, entdeckt werden (Abb. 2). Die Metaboliten wurden nicht identifiziert.

Mit den genannten Methoden wurden 20 Fälle von tödlichen Vergiftungen (im allgemeinen Suicide) untersucht. Die Resultate sind in Tabellen 1 und 2 angegeben. Der Anteil von Metaboliten war im allgemeinen viel niedriger als der des unveränderten Methaqualons, besonders wenn auch andere Arzneimittel oder Äthanol vorlagen. Bemerkenswert niedrige Konzentrationen von Metaboliten zeigte Fall Nr. 7 (Tabelle 2), in welchem auch hohe Konzentrationen von Salicylsäure gefunden wurden.

Im Harn wurden nur geringe Mengen von Methaqualon oder „freien" Metaboliten nachgewiesen, saure Hydrolyse setzte hingegen große Mengen „gebundene" Metaboliten frei. Im Blut oder in den Organen wurden nur unbedeutende Mengen von „gebundenen" Metaboliten entdeckt.

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Table 1. *Methaqualone and its main metabolites in material from five autopsy cases. No other drugs were detected, and ethanol concentrations in blood were less than 0.10%. The concentrations are expressed as mg of drug (or metabolite) per 100 g of tissue or body fluid*

Case No.	Method	Concentration in				
		blood	liver	kidney	urine	
					before hydrolysis	after hydrolysis
1	G	1.0	9.0	4.5	0	0
	T	1.2	4.0	5.0	+	15.4
	S	2.4	16.0	12.0	(+)	16.0
2	G	0.4	2.6	—	0.5	0.4
	T	(+)	(+)	+	+	12.5
	S	1.2	2.9	3.0	3.8	10–15
3	G	0.8	4.0	0.8	0	0
	T	—	—	—	—	++
	S	1.2	3.8	0.9	0	1.0
4	G	0.6	7.0	1.6	0.3	0
	T	—	—	—	—	—
	S	1.0	6.6	1.8	0.7	0
5	G	0.4	7.9	—	0	0.4
	T	+	+	—	+	20–25
	S	0.9	8.0	—	0	20.0

— = Not determined.

Methods of determination are: G = gas chromatography, includes only methaqualone.— T = thin-layer chromatography with either visual evaluation of the amounts of metabolites from the colored spots (where the signs (+)-+++ give the approximate relative concentrations) or ultra-violet spectrophotometry of the elueted metabolites (figures are given).— S = ultra-violet spectrophotometry, includes methaqualone + metabolites.

In der Zeit 1964 bis Juni 1970 wurde Methaqualon in 164 Fällen tödlicher Arzneimittelvergiftungen entdeckt. Der Anteil der Selbstmorde war etwa 90%. In 22 Fällen war der Tod offenbar das Resultat einer Intoxikation durch Methaqualon allein, in 7 fand man auch Äthylalkohol, in 48 andere Arzneimittel, die wahrscheinlich zum Tod beitrugen, und in 40 Fällen war die unmittelbare Todesursache keine Arzneimittelvergiftung.

Die Analysen wurden nach der Extraktion mit Hilfe der Ultraviolettspektrophotometrie ausgeführt.

Die Resultate der Analysen von der oben genannten ersten drei Gruppen sind in den Tabellen 3—7 wiedergegeben. Die Resultate der letzten Gruppe (andere Todesursache als Methaqualon) sind in Abb. 3 und 4 in Form von Diagrammen dargestellt. Der in den Abbildungslegenden vorkommende Q-Wert (0,4—0,7 und 1,1—2,7 mg Methaqualon in 100 ml Blut bzw. 100 g Leber) ist das „mittlere Intervall", erhalten durch Auslassen der niedrigsten und höchsten 25% aller Werte (Maehly, 1964). Als Vergleich wurde der entsprechende Q-Wert der Methaqualongehalte im Blut von 19 Kraftfahrern berechnet. Diese Personen wurden von der Polizei verdächtigt, ihre Fahrzeuge unter Einwirkung von Tabletten geführt zu haben. Der Q-Wert war in dieser Gruppe 0,5—0,8 mg Methaqualon per 100 ml Blut.

Bestimmte Grenzen der Methaqualonkonzentration im Blut, bei welchen eine ernsthafte Intoxikation eintritt, konnten mit Sicherheit nicht festgestellt werden; aus unserem Material scheint aber hervorzugehen, daß 1—2 mg Methaqualon per 100 ml Blut eine solche Grenze darstellt. Dabei ist zu bemerken, daß die Blutspiegel in Obduktionsmaterial wesentlich

Table 2. *Methaqualone and its main metabolites in material from 15 autopsy cases, where also other drugs and/or ethanol was detected. The concentrations are expressed as mg of drug (or metabolite) per 100 g of tissue or body fluid. (For abbreviations, see Table 1)*

Case No.	Method	Concentration in					Ethanol in blood (%)	Other drugs (name, concentration, material)
		blood	liver	kidney	Urine			
					before hydrolysis	after hydrolysis		
6	G	2.3	10.6	5.0	—	—	0	diazepam, 0.1, blood
	T	—	—	—	—	—		
	S	3.7	12.8	5.8	—	—		
7	G	2.4	15.3	4.3	0.8	0	0	salicylic acid, 12, liver
	T	+	+	+	(+)	+		
	S	3.0	16.0	4.7	0.8	0.7		
8	G	2.5	0	0	0	0	0.13	
	T	—	—	—	—	—		
	S	2.3	0	0	0	0		
9	G	1.5	7.0	1.3	—	—	0	diazepam, 0.1, blood
	T	(+)	(+)	—	—	—		
	S	1.9	7.0	1.5	—	—		
10	G	1.2	8.0	2.6	—	—	0	meprobamate, 10, liver
	T	—	—	—	—	—		
	S	1.5	10.0	3.0	—	—		
11	G	0.8	4.0	—	2.5	0	0.06	diazepam, 0.1, blood
	T	+	+	—	+	6		1.7, urine
	S	1.4	4.8	—	2.7	5–6		
12	G	0.5	2.7	1.2	0.2	0	0.14	penotobarbital, 5.7, liver
	T	—	—	—	—	—		
	S	1.0	3.6	1.5	0.4	0.3		

13	G	0.7	5.0	2.8	—	—	0	amitriptyline, 22, liver
	T	—	—	—	—	—		
	S	1.0	5.2	3.8	—	—		
14	G	0.7	8.0	1.6	0	0	0.01	pentobarbital, 3.9, liver
	T	—	—	—	—	—		meprobamate, 2.0, liver
	S	1.0	6.6	1.4	0	0		
15	G	0.4	2.2	0.4	0	0	0.27	
	T	—	—	—	—	—		
	S	0.9	2.4	1.0	0	0		
16	G	0.2	1.6	0.4	0	0	0	secobarbital + brallobarbital 1.1, blood
	T	+	+	+	++	+++		
	S	0.7	2.5	2.0	1.8	13.8		
17	G	0	0.6	0	0	0	0	meprobamate, 5.8, liver
	T	—	—	—	—	+		
	S	0.6	1.5	0.4	0	5.0		
18	G	0.3	1.5	—	0	0	0	amitriptyline, 2.5, liver
	T	—	—	—	—	—		
	S	0.6	2.3	—	0	2.0		
19	G	0.4	2.2	0	0	0	0	chlordiazepoxide, 0.2, blood
	T	—	—	—	—	—		
	S	0.6	2.5	0.4	0	0		
20	G	0	7.5	—	0	0	0.18	aprobarbital + vinbarbital, 2.0, liver
	T	—	—	—	—	—		
	S	0.5	6.4	1.0	0	0		

Table 3. *The concentration of methaqualone in autopsy material from 22 cases, where methaqualone evidently was the main cause of death (suicides)*

Year of death	Male/ female	Age (years)	Methaqualone (mg per 100 g)			Relevant autopsy findings
			blood	liver	kidney	
1969	f	35	2.4	16.0	12.0	
1968	f	48	2.2	—	—	
1968	f	30	4.0	11.5	—	
1970	m	50	2.7	—	—	
1968	f	33	0.5–1	6.0	—	
1970	m	28	—	5.8	—	
1967	m	49	0.6	5.5	1.6	heart failure, secondary bronchopneumonia
1968	f	54	1.1	5.5	—	heart failure, secondary bronchopneumonia
1967	m	28	1.1	5.0	2.3	
1967	f	82	1.1	—	—	heart failure
1964	m	53	1–2	4.7	4.0	
1970	f	28	—	4.0	—	
1965	f	39	1.2	3.9	2.6	
1969	m	69	—	3.3	—	
9169	m	26	1.2	3.2	—	
1965	m	68	0.6	3.1	2.0	secondary bronchopneumonia
1969	m	49	1.2	2.9	3.0	secondary bronchopneumonia
1969	m	69	—	2.9	—	
1970	m	46	—	2.6	—	
1969	f	69	—	2.4	—	
1969	f	68	1.0	2.1	—	
1970	m	63	—	0.94	—	

— = Not determined.

Table 4. *The concentration of methaqualone in 7 autopsy cases, where also ethanol (but no other drugs) was detected*

Year of death	Male/ female	Age (years)	Methaqualone (mg per 100 g)		Ethanol (% in blood)
			blood	liver	
1969	f	52	1–2	12.0	0.11
1967	m	29	1.5	7.0	0.08
1968	m	43	1.7	—	0.12
1969	m	55	1.0	6.6	0.07
1965	m	62	—	6.0	0.16
1969	f	39	0.9	2.4	0.27
1960	m[a]	33	0.5	2.3	0.15

— = Not examined.
[a] Chronic alcoholic.

niedriger sein können als in früheren Stadien der Vergiftung. Dies gilt besonders beim Eintreten von Komplikationen. Unsere Fälle mit solchen Komplikationen (häufig Pneumonie; s. Tabelle 3) zeigen eine Tendenz gegen niedrigere Konzentrationen; da aber das Material zu wenig umfangreich ist, kann diese Tendenz nicht mit Sicherheit bestätigt werden.

Table 5. *The concentration of methaqualone and short-acting barbiturate hypnotics*[a] *in 15 cases, where an intoxication with this drug combination evidently was the cause of death*

Year of death	Male/ female	Age (years)	Methaqualone (mg per 100 g)			Ethanol (% in blood)	Barbiturate (mg per 100 g)		Other drugs (mg per 100 g)
			blood	liver	kidney		blood	liver	
1968	f	20	2.1	—	—	—	2.5	—	meprobamate, 9.0, blood
1969	m	61	1.5	10.0	3.0	0	—	4.5	meprobamate, 10.0, liver
1969	m	49	1.0	6.5	1.4	0.01	—	3.9	meprobamate, 2.0, liver
1969	m	28	1.0	3.6	1.5	0.14	—	5.7	
1966	f	59	1.2	3.3	—	—	2.0	4.9	
1969	m	57	—	2–4	—	0	—	2.2	
1967	f	42	0.5	3.0	—	—	0.9	2.3	
1965	m	43	—	2.0	—	0.17	1.4	4.0	
1969	m	52	—	2.0	—	0.06	—	5.7	phenothiazine derivative 0.6, liver
1969	m	49	0	2.0	—	0	—	21.0	
1967	m[b]	29	0.5	1.4	—	—	0.3	0.4	
1968	f	52	0.5	—	—	—	2.0	—	
1965	f	55	0.2	0.8	—	0.23	1.2	10.0	
1969	m	53	—	0.7	—	0	—	8.9	
1968	m	47	—	0.5	—	0.06	—	36.0	

0 = Not detectable.——— = Not determined.

[a] Pentobarbital, amobarbital.—[b] Secondary bronchopneumonia.

Table 6. *The concentration of methaqualone and medium-acting barbiturate hypnotics*[a] *in 14 cases, where an intoxication with this drug combination evidently was the cause of death*

Year of death	Male/ female	Age (years)	Methaqualone (mg per 100 g)			Ethanol (% in blood)	Barbiturate (mg per 100 g)		Other drugs (mg per 100 g)
			blood	liver	kidney		blood	liver	
1969	f	29	—	8.0	—	0	—	25.0	
1965	m	26	—	6.7	1.4	—	—	15.0	meprobamate, 30.0, liver
1969	m	47	0.5	6.4	1.0	0.18	—	2.0	
1964	f	47	3.1	5.5	5.1	0	3.2	4.5	
1966	m	44	1.6	—	—	0.13	2.1	—	
1968	f	67	0.5	—	—	—	3.6	—	meprobamate, 11.0, blood
1968	m	36	0.4	—	—	0.10	1.0	—	
1965	f	32	0.4	5.3	5.0	0	1.4	7.5	
1969	m	48	0.7	2.5	2.0	0	1.1	1.0	
1969	m	34	0.3	1.6	1.0	0	—	6.5	
1969	m[b]	52	—	1.5	—	0.16	—	9.0	
1969	m	38	—	1.4	—	0	1.6	10.0	
1968	m	49	—	1.0	—	0.05	—	15.0	
1969	m	45	0.3	1.0	—	—	3.5	7.2	

0 = Not detectable.—— = Not determined.

[a] Aprobarbital, vinbarbital, secobarbital, brallobarbital.—[b] heart failure.

In Fällen, in denen außer Methaqualon auch Äthylalkohol vorkommt, sind die beobachteten Konzentrationen des Arzneimittels nicht wesentlich niedriger (Tabelle 4; *Q*-Wert 0,9 bis 1,5 mg per 100 ml Blut) als bei „reinen" Vergiftungen (Tabelle 3; *Q*-Wert 1,1—1,5), aber hier genügt der Umfang des Materials nicht für einen sicheren Schluß.

Aus den Tabellen 5 und 6 geht hervor, daß die gleichzeitige Zufuhr von Barbitursäure-Schlafmitteln die Toxicität des Methaqualons steigert.

Zahlreiche Methaqualonvergiftungen sind in der Literatur beschrieben, aber die Konzentrationen dieses Arzneimittels in Blut oder Organgeweben wurden verhältnismäßig selten gemessen, und die Grenzen der lebensbedrohenden Konzentrationen wurden sehr verschieden angegeben.

The hypnotic action of methaqualone (2-methyl-3-*o*-tolyl-4(3H)-quinazolinone) was first demonstrated in 1955. It is now widely used, like the barbiturates, for producing sleep. Intoxications by overdose do not seldom occur, and there are numerous reports about self-poisoning.

According to Bünger *et al.* (1964), 67 out of 611 cases of deliberate drug overdosages (11%) occurring in Hamburg during the time 1962–1963 involved methaqualone. Ibe (1965) reports 69 methaqualone poisonings in Berlin during 1960 to 1963, Maehly and Bonnichsen (1966) reported 5 fatal cases in Sweden, and Burston (1967) found that 15% of attempted suicides with drugs were caused by Mandrax® (methaqualone + diphenhydramine) during the first quarter of 1967. Corresponding values were found by Lawson and Brown (1967) to be 5% in Scotland (during part of 1965), and by Proudfoot *et al.* (1968) to be 8.5% in Edinburgh (during part of 1966).

At least 20 fatal poisonings with methaqualone—partly in combinations with other drugs—and a great number of serious but non-fatal poisonings have been described in the literature (Schmitt, 1962; Geldmacher-Mallinckrodt and Lautenbach, 1963; Ibe, 1965; Sanderson *et al.*, 1966; Caridis *et al.*, 1967; Ford and Birt, 1967; Gitelson, 1967; Knoke and Schlabitz, 1967; Lawson and Brown, 1966, 1967; Wilkinson, 1967; Tompsett, 1968; Proudfoot *et al.*, 1968; and Heyndrickx and de Leenheer, 1969). Of these authors, Geldmacher-Mallinckrodt and Lautenbach (1963) and Tompsett (1968) performed analyses on autopsy material.

Various clinical and chemical data on cases of methaqualone poisonings are given by Ibe (1965, 1966a, b), Lawson and Brown (1966, 1967), Caridis *et al.* (1967), and Matthew *et al.* (1968).

This contribution presents a limited number of autopsy cases, which are toxicologically analyzed in a detailed fashion, and a statistical survey of a large number of methaqualone poisonings which are more routinely investigated.

Material and Methods

The autopsy material was routinely analysed for hypnotic drugs according to the methods of Bonnichsen *et al.* (1961) and Maehly and Bonnichsen (1966). Since 1968 the procedure was somewhat modified in the cases of tissue analyses, involving a preliminary extraction with ethanol (see procedure). Twenty cases were given a more detailed examination including thin-layer and gas chromatographic as well as spectrophotometric methods.

Apparatus and Reagents. For spectrophotometric determinations in the ultra-violet region, a Unicam SP 800 double beam recording instrument was used. The gas chromatograph used was a Varian 1200 instrument with a glass column, 5 feet in length and of 1.5 mm I.D. ($^1/_4$″ O.D.). The column packing was 1% HiEff 3A (neopentyl glycol adipate from Applied

Table 7. *The concentration of methaqualone and drugs (other than short- and medium-acting barbiturates) in 19 cases, where an intoxication with combinations of drugs evidently caused the death*

Year of death	Male/ female	Age (years)	Methaqualone (mg per 100 g)			Ethanol (%)	Other drugs (mg per 100 g)				Additional autopsy findings
			blood	liver	kidney		blood	liver	kidney	urine	
1969	m	27	3.0	16.0	4.7	0	Salicylic acid				
							—	12.0	—	—	
1969	m	35	3.7	12.8	5.8	—	Diazepam				
							0.1	—	—	0.6	
1969	m	41	—	7.5	—	0.10	Phenobarbital				
							—	36.0	—	—	
1969	f	58	1.9	7.0	1.5	0	Diazepam				
							0.1	—	—	—	
1969	f	60	1.0	5.2	3.8	0	Amitriptyline				
							—	22.0	—	—	
1968	f	48	0.9	5.0	—	—	Hexapropymate				
							1.2	6.2	—	—	
1969	m	40	1.4	4.8	—	0.06	Diazepam				
							0.1	—	—	1.7	
1968	m	47	1.6	4.8	—	—	Chlordiazepoxide				
							—	—	—	0.4	
1968	f	63	—	3.5	—	0	Ethchlorvynol				
							19.5	—	—	—	

1968	m	70	—	3.0	—	0	Salicylic acid				heart failure
							—	10.3	—	—	
1968	f	30	—	2.5	—	—	Levomepromazine				
							—	4.3	—	—	
1969	m		0.6	2.5	0.4	0	Chlordiazepoxide				
							0.2	—	—	—	chronic alcoholic
1969	f	15	0.6	2.3	—	—	Amitriptyline				
							—	2.5	—	—	
1968	m		0.7	2.1	—	0.30	Diazepam				
							—	—	—	0.1	
1967	f	44	1.0	2.0	—	—	Meprobamate				
							6.5	5.6	—	—	
1968	m	50	—	1.5	—	0.19	Phenobarbital + cyclobarbital				
							—	4.1	—	—	
1969	f	31	0.3	1.5	—	0.05	Benzodiazepine derivative				
							—	—	—	0.04	
1969	m	47	0.2	1.4	—	0.20	Meprobamate				chronic alcoholic;
							0.1	—	—	0.5	aspiration of gastric contents
1967	m	37	0.6	0.5	—	0.18	Phenobarbital				chronic alcoholic;
							0.3	0.8	—	—	accidental overdosage

0 = Not detectable.—— = Not determined.

Science Laboratories, Pa., U.S.A.) on Gas Chrom Q (100–120 mesh). The temperatures were: oven 190°, injector and detector (flame ionization) 260°. The flowrates of nitrogen and hydrogen were adjusted to about 30 ml/min for each.

For thin-layer chromatography, plates were prepared according to Stahl (1967) with MN Kieselgel G/UV_{254} (silica gel from Macherey, Nagel & Co., Düren, Germany). The spotted plates were developed with chloroform—acetone (9+1). The compounds were detected by inspection in ultra-violet light and by spraying with Dragendorff's reagent (Curry, 1960). The reagent gave orange-red spots with methaqualone and some of its metabolites (Fig. 2).

All reagents were of analytical grade purity except the chloroform, which was of technical grade and redistilled over anhydrous calcium chloride prior to use.

Dilute ammonia in ethanol: to 780 ml of ethanol (95%), 2.0 ml of concentrated ammonia (25%) was added, and the solution was diluted to 1000 ml with water.

Procedure. Urine or blood was extracted according to Bonnichsen *et al.* (1961) and Maehly and Bonnichsen (1966). In the case of tissues, a preliminary extraction was performed with ethanol (Bonnichsen, 1970): pieces of liver or kidney (30 g) were homogenized with 20 ml of water in a Polytron PD-35 OD homoginizer (Kinematica GmbH, Luzern, Switzerland). After the addition of 125 ml of 95% ethanol, the slurry was further homogenized and then left over night at +4°. The weight of the suspension was adjusted to 150 g by the addition of ethanol and centrifuged. After a new weight adjustment the supernatant was decanted off, and 50 g of the clear solution was evaporated over a boiling water bath under a stream of air until a few milliliters were left. The residue was then treated with hot 0.01 N hydrochloric acid and filtered in the cold (in order to remove lipids and some other interfering substances) according to Bonnichsen *et al.* (1961). The remainder of the extracting procedure was performed as reported in the last-mentioned paper.

The resulting extracts were directly subjected to gas chromatography. For examination by ultra-violet spectrophotometry, an aliquot of the extract was shaken with four volumes of 0.5 N ammonia to remove acidic impurities. The chloroform layer was evaporated to dryness, the residue was dissolved in a known volume of dilute ammonia in ethanol, and the spectrum was recorded. In addition, thin-layer chromatography was performed in some of the cases investigated. From visual inspection, a merely approximate estimation of the amounts was carried out. For more accurate determinations, spots were eluted and the eluates examined by ultra-violet spectrophotometry after redissolution in dilute ammonia in ethanol. The same absorption coefficients as for the parent drug were used for quantitative analysis (Nowak *et al.*, 1966).

For determination of conjugated metabolites, a previously extracted urine aliquot was heated with an equal volume of concentrated hydrochloric acid (37%) to 100° for an hour. After cooling, extraction was carried out as described above.

Results and Discussion

The Analytical Method. In earlier published investigations, methaqualone was usually assayed by ultra-violet spectrophotometry after extraction. Various extraction procedures have been employed (Preuss *et al.*, 1966a, b; Nowak *et al.*, 1966; Geldmacher-Mallinckrodt and Lautenbach, 1963; Heyndrickx and de Leenheer, 1969; and others). In the extraction procedure used in this investigation, the recovery of added methaqualone was about 85%.

The ultra-violet spectrum of pure methaqualone solution is shown in Fig. 1. Three different ways for determining the absorbancy are designated as I, II and III. Usually, II has been employed for determination of the concentration, but sometimes interfering substances make it more convenient to use III. The construction I has a marked tendency to give too high values in extracts from biological material, except in cases with very high concentrations of the drug.

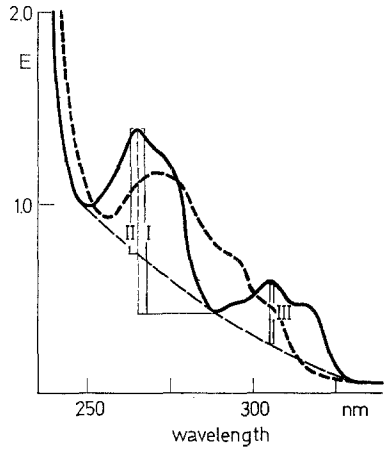

Fig. 1

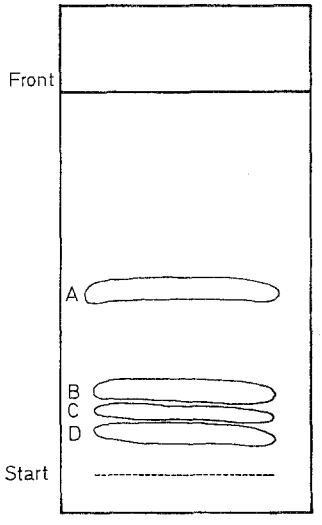

Fig. 2

Fig. 1. Ultra-violet absorption spectrum of methaqualone (40 μg/ml) in dilute ammonia in ethanol ——. The same solution after the addition of 6 N sulfuric acid to acidic reaction — — —. *I*, *II* and *III* denote different ways of measuring the absorbance: *I* is the difference between the maximum at 265 nm and the minimum at 288 nm, while *II* and *III* are the peak heights at 265 nm and 305 nm, measured from straight base-lines drawn through the minima at 248–288 nm and 288–328 nm, respectively

Fig. 2. Case no. 1 (Table 1). TLC from extract of kidney. The areas marked are ultra-violet absorbing and give orange-red color with Dragendorff's reagent. The spot A corresponds in respect to the R_f value to methaqualone, the spots B, C and D represent metabolites of methaqualone. Conditions for the chromatography, see p. 343

Occurrence of Metabolites. Several metabolites of methaqualone have been reported to appear in the urine after the administration of this drug. Some of them are extracted under the same conditions as the parent drug, and they also exhibit similar ultra-violet absorption spectra (Preuss *et al.*, 1966b). In order to perform a differentiation between parent drug and its metabolites, various authors employed paper or thin-layer chromatography (Preuss *et al.*, 1966a, b; Nowak *et al.*, 1966). In the present investigation, a procedure for differentiation between methaqualone and its main metabolites, suitable for routine analyses, was adopted. This procedure involved spectrophotometric and gas chromatographic examination of the extracts. With gas chromatography, carried out under the conditions given under "material and methods", only methaqualone itself is determined (it formed a peak with a retention time of about 8 minutes—the metabolites were not eluted), while spectrophotometry allows for the determination of both methaqualone and its metabolites.

The data obtained from the cases, which underwent a more detailed analytical procedure, are presented in Table 1 and 2. It is seen that the ratio of the concentration of the metabolites to that of methaqualone itself in blood, liver and kidney varies much from one case to another. In a few cases this ratio is about unity

(*e.g.* case no. 1), but in most cases the metabolite concentrations are considerably lower, especially when ethanol or other drugs are present (Table 2). Of special interest is case no. 7 (Table 2), where salicylic acid also was found. In blood, liver, kidney and also urine of this case, the metabolite concentrations were very low in comparison to the concentration of methaqualone. This could be due to a competitive action of salicylic acid on the enzyme systems, responsible for the metabolism of methaqualone. (At present, investigations of the influence of salicylic acid on the metabolism of various drugs are being carried out at this laboratory).

Usually, very low concentrations of methaqualone and also of "free" metabolites are present in the urine—in agreement with earlier observations on methaqualone excretion in man and some animals (Beyer, 1965; Beyer and Klinge, 1964; Preuss *et al.*, 1966a, b; Nowak *et al.*, 1966). However, large amounts of metabolites are liberated after hydrolysis, with R_f values on thin layers and with ultra-violet spectra approximately corresponding to those of the "free" metabolites found in blood and tissues (see Fig. 2). Preuss *et al.* (1966a, b) detected eight such metabolites in human urine, and they proved the identity of four of these. To our knowledge, no "free" metabolites from urine, nor any metabolites from tissues, have been identified. "Free" urinary metabolites may differ from "bound" metabolites, as the former are reported not to react with diazotized sulfanilic acid, while most of the "bound" ones do react (Preuss *et al.*, 1966b).

In some instances—case no:s 1, 7, 8 and 14—the aqueous residue remaining after the extraction of blood and liver were hydrolyzed as previously described for urine, and then extracted. No significant amounts of methaqualone or its metabolites were detected.

In the present investigation, no efforts have been undertaken to identify the metabolites. Also the nature of the conjugates are to some extent still an open question—according to Beyer and Klinge (1964), considerably greater amounts of extractable metabolites are formed after acid hydrolysis than after enzymatic hydrolysis with β-glucuronidase. This indicates the occurrence of other conjugates than glucuronides *e.g.* ethereal sulfates. A mixture of β-glucuronidase and sulfatase liberated a considerably larger amount of metabolites, but 20% still remained unextracted, as compared with acid hydrolysis (Beyer, 1965).

Methaqualone Concentrations in Autopsy Material. During the time January, 1964 to June, 1970, methaqualone was detected in 165 autopsy cases (2 in 1964, 7 in 1965, 5 in 1966, 16 in 1967, 38 in 1968, 69 in 1969 and 28 in the time January–June, 1970). Of these cases, all but 48 detected during the last period of time (October, 1969 to June, 1970), are presented in the following. However, "pure" methaqualone poisonings from the entire period is presented (Table 3).

In 22 instances poisoning was probably due to methaqualone alone, in 7 cases ethanol was also present, and in 48 cases other drugs were found as well, probably contributing to death. In 40 autopsies, the immediate cause of death was judged to be another than an overdosage of drugs.

A marked increase in the incidence of self-poisoning by methaqualone has been reported from several countries. This also seems to hold true for Sweden, as indicated by the results reported here.

The results of the chemical analyses are presented in Tables (3–7) and in the form of diagrams (Figs. 3 and 4). (The cases in Table 1 and 2 are included in the

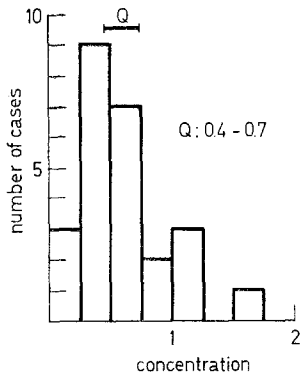

Fig. 3

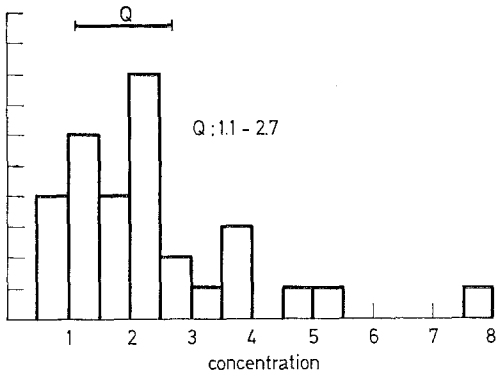

Fig. 4

Fig. 3. The concentration of methaqualone in blood (mg per 100 ml) in 25 out of 40 cases where death was caused by other factors than drugs. *Q* represents the concentration range within the "average" group, obtained by leaving out the lowest and highest 25 per cent of all cases investigated

Fig. 4. The concentration of methaqualone in liver (mg per 100 g) from 31 out of 40 cases where death evidently was caused by other factors than drugs

statistical material.) In each table the cases as a rule are arranged in the order of decreasing methaqualone concentration in the liver. Table 3 includes the first mentioned group of "pure" methaqualone poisonings, and Table 4 gives the corresponding data for cases where methaqualone and ethanol (but no other drugs) were detected. Table 5 and 6 include cases where short- and medium-acting barbiturate hypnotics were detected as well, and Table 7 lists instances where methaqualone in combination with other drugs evidently caused death.

The remaining group (where death were not caused primarily by methaqualone–most victims of this group died from drowning) is presented as diagrams of methaqualone concentration in blood (Fig. 3) and in liver (Fig. 4), respectively. The concentration range *Q* was determined as described by Maehly (1964) and found to be 0.4–0.7 and 1.1–2.7 mg per 100 ml of blood and per 100 g of liver. For comparison the blood concentration of methaqualone in 19 car drivers, suspected by the police of having been under the influence of drugs, were calculated with respect to the *Q* value. The resulting *Q* value was 0.5–0.8 mg per 100 ml of blood.

Concerning the lower limit of "lethal concentration" of methaqualone in blood and liver, very little can be learned from the literature. Geldmacher-Mallinckrodt and Lautenbach (1963) found 2.7 mg, and Tompsett (1968) found 31 mg of methaqualone per 100 g of liver in two fatal cases. There is, however, more information about results from clinical analyses of poisoning cases. Ibe (1965) found in two survived cases 1.4 mg per 100 ml of plasma and 9.0 mg per 100 ml of blood, respectively, and in a fatal case he found 3.6 mg per 100 ml of blood. Lawson and Brown (1966, 1967) reported values from 0.9 to 4.7 mg per 100 ml of plasma in cases of serious intoxications and conclude that a plasma level of 2.5–3.0 mg per 100 ml indicates a critical poisoning. In contrast to these figures, Matthew *et al.* (1968) found 27 out of 42 patients with plasma levels of 2.5 mg per 100 ml or more who were only moderately intoxicated.

It can be mentioned for comparison that plasma levels of methaqualone in 15 patients on therapeutic regimes of Mandrax® about 12 hours after ingestion were found to range from 0.09–0.22 mg per 100 ml (Brown and Smart, 1969).

With intensive treatment even patients with high concentrations of methaqualone may survive. Thus, Caridis *et al.* (1967) report a successful treatment of a patient with as much as 10.5 mg methaqualone per 100 ml of plasma, whereas Proudfoot *et al.* (1968) present a case of a patient with 23 mg per 100 ml of plasma who died in spite of intensive treatment (peritoneal dialysis).

From the present report, definite conclusions cannot be drawn about the limit of "lethal concentrations" of methaqualone in blood or liver. It seems that values of 1—2 mg per 100 ml of blood may represent the danger limit, while "survival concentrations" (drugged drivers and autopsy cases, where methaqualone was supposed to have only minor effects) lie at about 0.4–0.7 mg per 100 ml of blood. Thus, the difference between "lethal" and "survival" concentrations does not seem to be very great.

The "lethal concentrations" of methaqualone in blood are thus of the same order as the corresponding figures for barbiturate hypnotics of short-acting duration (such as pentobarbital and amobarbital; Bonnichsen *et al.*, 1961). However, it must be noted that methaqualone concentrations at death, *i.e.* in autopsy material, would be lower, sometimes considerably lower, than they would be during earlier stages of the poisoning process. It is probable that many individuals died when large amounts of the drug had already been metabolized and excreted.

The lower limit of "lethal concentrations" of methaqualone in liver seems to be about 3–4 mg per 100 g. Thus, a very small difference exists between "lethal" and "survival" concentrations in the liver, as the Q value of the latter amounts to 1.1–2.7 mg per 100 g (Fig. 4). In 10 cases of drowning the liver concentrations ranged from 0.5–4.8 mg drug per 100 g of liver. However, it is not known to what extent methaqualone and its metabolites accumulate in the liver during prolonged use of the drug.

An important fact is that the spectrophotometric measurements also include some metabolites of methaqualone. According to Nowak *et al.* (1966), these metabolites are less toxic than the parent drug, and a better knowledge of the relation between parent drug and metabolites would be of great value for evaluating the intoxications by this drug. The metabolism may be influenced by the presence of other drugs and by other factors such as genetically dependant hypo- and hyperactivity of the appropriate enzyme systems, or repeated exposition of the organism to methaqualone or other drugs.

Table 4 was set up to show the possible influence of ethanol on the toxicity of methaqualone. The Q values (definition, see legend to Fig. 3) derived from this table amounted to 0.9–1.5 and 2.4–7.0 mg methaqualone per 100 ml of blood and 100 g of liver, respectively. Thus, from Table 4 it does not seem that ethanol increases the toxicity of methaqualone. However, such a tendency is indicated in Table 7 (including also other drugs—see below). This is an important point, as it is sometimes assumed that ethanol increases the toxicity of methaqualone as it does *e.g.* that of barbiturate hypnotics (Bonnichsen *et al.*, 1961), but to the authors' knowledge, no published investigation on the interaction between methaqualone and ethanol has appeared in the literature.

Very divergent concentrations of methaqualone were found in cases where other drugs were also present. The cases in Table 5 and 6 (combinations with barbiturate hypnotics of short and medium duration, respectively) exhibited as a rule high methaqualone concentrations if the barbiturate concentrations were low and *vice versa*. In fact, the cases with the lowest methaqualone concentrations presented sufficiently high barbiturate levels to be lethal by themselves.

Table 7 presents fatal poisonings with combinations of methaqualone with various drugs, other than short- and medium-acting barbiturates. It seems that in cases with high methaqualone concentrations, high concentrations of other drugs also exist. A possible explanation of this observation might be that the metabolism of methaqualone is inhibited by the presence of other drugs (competitive action on certain enzyme systems), so that a larger proportion is excreted in unchanged form.

The last four cases in Table 7 show only low concentration of drugs but rather high ethanol values—which indicates a potentiating effect of ethanol on methaqualone toxicity.

Secondary causes such as pneumonia will often contribute to death, as aspiration is a common complication in poisoning with methaqualone—this drug produces abundant secretions from the upper respiratory and digestive tracts (Ibe, 1965, 1966a; Holmberg and Wiklund, 1970, *loc. cit.*). Important observations of this nature found at autopsy are reported in the tables. Table 3 indicates that the victims exhibiting such changes as a rule had lower methaqualone concentrations than the remaining cases in the table, but the material is not sufficient to allow a relevant conclusion.

Since methaqualone concentrations based on ultra-violet spectra can be calculated in more than one way, values from different authors are not quite comparable. For example, the values given in this paper tend to be lower than those of Maehly and Bonnichsen (1966), especially when impurities are present.

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Addendum. During the preparation of this paper, an investigation was published, in which the concentration of methaqualone in the plasma of human subjects after the intake of a single dose (150 or 250 mg) was determined. The level reached a maximum after about one hour and amounted 0.1—0.2 mg per 100 ml [Berry, D. H. J.: Gas chromatographic determination of methaqualone, 2-methyl-3-o-tolyl-4(3H)-quinazolinone, at therapeutic levels in human plasma. J. Chromatog. **42**, 39—44 (1969)].

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