The lipid composition of epididymal fat pad in different groups are shown in Table II. There was significant reduction of total lipids in the saline-treated diabetic group which was mainly due to reduction in triglyceride fraction. Significant decrease in esterified cholesterol and increase in phospholipid and free fatty acid has been observed in this group. With insulin treatment, total lipid, neutral lipid and triglyceride were restored to the values observed in saline treated control group. There was no effect of insulin on esterified cholesterol, while free fatty acid increased further. In the non-diabetic animals, continued insulin treatment caused an increase in the total lipid which was mainly due to increase in triglyceride fraction. Slight though significant decrease was also seen in free fatty acid level in insulin treated non-diabetic animals.

Table II. Adipose tissue lipids in various groups

Lipids (mg/g)	Saline treated control	Insulin treated control	Saline treated diabetic	Insulin treated diabetic
Total lipid	715±26.5	812±7.6 $p<0.005$[a]	595±4.6 $p<0.005$[a]	718±31 $p<0.001$[b]
Neutral lipid	713±24	810±7.6 $p<0.005$[a]	592±5.7 $p<0.005$[a]	714±31.4 $p<0.001$[b]
Phospholipid	2.49±0.21	2.58±0.04	3.66±0.15 $p<0.001$[a]	3.92±0.39 $p<0.01$[a]
Triglyceride	452±8.6	518±9.2 $p<0.001$[a]	345±9.4 $p<0.001$[a]	471±9.2 $p<0.001$[b]
Total cholesterol	6.38±0.69	7.05±0.1	4.28±0.48 $p<0.02$[a]	4.44±0.48 $p<0.05$[a]
Free cholesterol	3.29±0.35	3.85±0.12	2.88±0.39	2.94±0.38
Esterified cholesterol	3.09±0.38	3.14±0.07	1.35±0.05 $p<0.001$[a]	1.35±0.13 $p<0.01$[a]
Free fatty acid	3±0.15	2.3±0.1 $p<0.005$[a]	4.8±0.2 $p<0.001$[a]	7.6±0.25 $p<0.001$[a] $p<0.001$[b]

Values were Mean ± SE of 5 observations in each group
[a] As compared to saline treated controls
[b] As compared to saline treated diabetics

It has been reported that adipose tissue clearing lipase is low in alloxan diabetic rats[11,12]. At the same time, there is increased mobilization of free fatty acids from the adipose tissue in diabetes[13]. These factors might have contributed to the decreased lipid content of the adipose tissue in chronic diabetic rats. Insulin administration restored the lipid content of the adipose tissue of the diabetic animal, possibly due to enhanced lipogenic effect and decreased lipolytic effect of the hormone[14]. Hyperinsulinism has been involved with such risks as obesity[15], with or without glucose intolerance[16] and hypertriglyceridemia[17]. The long-term in vivo effect of hyperinsulinism on adipose tissue lipid metabolism in normal non-diabetic animals has received relatively little attention in the past. From the results of the present investigation, it is clearly evident that long-term insulin administration leads to increased lipid accumulation in the adipose tissue.

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MAO inhibition, an unlikely mode of action for chlordimeform

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Summary. Inhibition constants of several formamidines, their corresponding formanilides and other representatives of compounds derived from aniline, such as phenylureas, N-phenyl-carbamates and acylanilides, were determined for rat liver monoamine oxidase. The reversability of the inhibition and the lack of correlation between inhibition potencies and toxicities of the compounds tested add to the opinion that MAO inhibition is not a prominent factor in chlordimeform poisoning.

The insecticide/acaricide/ovicide chlordimeform, N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine, and its N-demethyl derivative are known to inhibit monoamine oxidase (MAO) in homogenates of rat liver[1,2], cattle ticks[3,4] and cockroach heads[5]. Whereas the first publications[1,2] attempted to illustrate the importance of this inhibition with regard to the biochemical mode of action of chlordimeform, subsequent papers did only express some[3,5] or no primary involvement[4] of MAO inhibition in the lethal action of formamidines.

All inhibition data published so far are I_{50}-values obtained from pre-incubation experiments. The type of inhibition, however, as compared to that of known inhibitors of pharmacological importance has not yet been evaluated and, with exception of the N-demethyl derivative, no other metabolite of chlordimeform was tested for its inhibition potency. In addition, the I_{50}-determinations did not consider further types of pesticides con-

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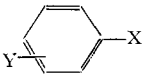
taining the aniline moiety, such as phenylureas, phenylcarbamates and acylanilides. Therefore, our objective was to extend the previous inhibition studies to compounds more or less related to formamidines in order to gain some insight into structural requirements of MAO inhibitors.

Materials and methods. The inhibition experiments were carried out with a mitochondria containing fraction (1000–10,000 × g) of rat liver homogenates ($^1/_{15}$ M Soerensen phosphate buffer pH 8.0). In most of the tests kynuramine was used as a substrate[6], but K_i-values for chlordimeform were also determined in the presence of serotonin, tyramine, tryptamine, and β-phenylethylamine by means of an oxygen electrode. The reaction was started by adding the enzyme to the substrate-inhibitor mixture.

Results. The formamidines and formanilides were found to be relatively potent, but reversible and competitive inhibitors of rat liver MAO. This mode of inhibition contrasts with that of many drugs with an irreversible anti-MAO activity, such as pargyline, clorgyline, iproniazid, tranylcypromine, nialamide, phenelzine and others. Therefore, chlordimeform in its mode of inhibition may be more comparable to a reversible inhibitor, such as procaine, which has been characterized by an absence of severe adverse reactions that are traditionally associated with irreversible MAO-inhibitors[7].

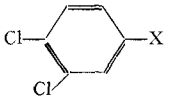
Table 1. Inhibition constants (K_i-values) of various formamidines and formanilides determind for rat liver MAO catalyzed oxidation of kynuramine



Y =	X = –N=CH–N(CH$_3$)$_2$	X = –NH–CHO
H	18	37
2-CH$_3$	7.7	4.0
2,4-diCH$_3$	3.1	3.5
4-Cl	7.4	2.5
3,4-diCl	2.4	0.35
2-CH$_3$,4-Cl	2.7	1.3
2-C$_2$H$_5$,4-Cl	8.6	2.2

Inhibition constants are expressed as μM.

Table 2. Inhibition constants (K_i-values) of 3,4-dichloro substituted formanilide, propionanilide, methyl-N-phenylcarbamate, N-phenyl-N′, N′-dimethylurea, and N-phenyl-N′, N′-dimethylformamidine



X =	Common name	K_i-value (μM)
–NH–C(O)H		0.35
–NH–C(O)CH$_2$CH$_3$	Propanil	2.0
–NH–C(O)OCH$_3$	Swep	0.85
–NH–C(O)–N(CH$_3$)$_2$	Diuron	3.4
–N=CH–N(CH$_3$)$_2$		2.4

Table 3. Toxicities expressed as the acute oral LD_{50} for rats of chlordimeform, its formyl-metabolite, and the herbicides propanil, swep and diuron

Compound	LD_{50} (mg/kg)	Ref.
Chlordimeform	340, 400	12, 11
N-Formyl-4-chloro-o-toluidine	2900	11
Propanil	1384	12
Swep	552	12
Diuron	3400	12

The inhibition constants (K_i-values) of seven formamidines and their corresponding formanilides (table 1) confirm earlier findings[1,2] that the 4-chloro-o-tolyl moiety of chlordimeform is associated with a considerable inhibition potency in vitro. The K_i-values of the 3,4-dichloro and the 2,4-dimethyl derivatives were in the same range as that of chlordimeform. Surprisingly, almost all formyl derivatives tested have inhibition constants that are even lower than those of the parent compounds but, at the same time, are known to be without any insecticidal/acaricidal/ovicidal effect[8–11]. Even 4-chloro-o-toluidine, a minor metabolite of chlordimeform, acted as an anti-MAO agent under our test conditions, the K_i-value being 9 μM.

The determination of K_i-values of chlordimeform with several MAO substrates (Clark oxygen electrode) resulted in the following constants: with kynuramine 3.6, with serotonin 3.0, with tyramine 1.9, with β-phenylethylamine 3.9, and with tryptamine 6.9 μM. Thus, the K_i-values are only poorly dependent on the substrate used, and chlordimeform cannot differentiate between the types A and B of MAO.

The low K_i-values, i.e. the high inhibition potencies of the formanilides made us then investigate a number of additional analogs in which the hydrogen attached to the carbonyl group was replaced by an alkyl (ethyl), an alkoxy (methoxy), or a dimethylamino moiety. The resulting compounds are an acylanilide, a N-phenylcarbamate, and a phenylurea, respectively. With the 3,4-dichloro substitution in the phenyl portion of the molecule the well known herbicides propanil, swep and diuron are obtained. The K_i-values determined for this series of compounds are listed in table 2. Again, the formanilide was found to be the best inhibitor, followed by swep, propanil, the fromamidine and finally diuron.

The above findings clearly demonstrate that the in vitro inhibition of MAO by chlordimeform is not a structure-specific phenomenon. Other types of pesticidal compounds carrying the aniline moiety inhibit rat liver MAO as well, some of them even better than chlordimeform, although the mammalian and insect toxicities of the herbicides mentioned are much more favorable than the toxicity of the insecticide/acaricide/ovicide chlordimeform (table 3). This lack of correlation between enzyme inhibition and toxicities appears to be an additional argument against the MAO hypothesis for both insects and mammals. Thus, we agree with Holden and Hadfield[4] who, on the basis of their recent studies with insects and ticks, concluded that MAO inhibition is not the cause of death of chlordimeform poisoning.

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