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ACUTE TOXICITY AND CHOLINESTERASE INHIBITION IN VIVO OF CHLORTHIOPHOS*

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The acute toxicity of chlorthiophos with a chief component 0,0-diethyl-0-[2,5-dichloro-4-(methylthio) phenyl] thionophosphate, combined with 0,0-diethyl-0-[4,5-dichloro-2-(methylthio) phenyl] thionophosphate and 0,0-diethyl-0-[2,4-dichloro-5-(methylthio) phenyl] thionophosphate is described. The oral LD_{50} values obtained are: rat, 13 mg/kg; mouse, 141 mg/kg; guinea-pig, 58 mg/kg; rabbit, 20 mg/kg; quail, 45 mg/kg; hen, 45 mg/kg, and dermal LD_{50} for: rat, 58 mg/kg and for rabbit, 48 mg/kg. No signs of neurotoxicity in hens were observed. In rats, a maximal inhibition of erythrocyte cholinesterase was found after two hours, of plasma cholinesterase after four and of brain cholinesterase after six hours, with a reversal to normal in the blood observed after four days and in brain after eight days. Lindane and phenobarbital pretreatment reduced the acute toxicity of chlorthiophos. Atropine and some oximes (obidoximechloride and trimedoximebromide) antagonized the toxic effects of chlorthiophos.

In earlier publications we reported on bromophos (1, 2) and bromophos-ethyl (3), a dimethyl – and diethylphosphorothioate. The present paper deals with the acute toxicity of chlorthiophos (Celamerck S 2957). Its aim is to facilitate the use of the substance.

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* Chlorthiophos is the proposed common name for Celamerck S 2957 (proposed commercial name: Celathion).

Some details concerning suitable antidotes in animal experiments will be helpful in the case of intoxication by chlorthiophos. Chlorthiophos synthetized by *Sehring* (4,5) is a 95% pure experimental insecticide. Its chief component is 0,0-diethyl-0-[2,5-dichloro-4-(methylthio) phenyl] thionophosphate, combined with 0,0-diethyl-0-[4,5-dichloro-2-(methylthio) phenyl] thionophosphate and 0,0-diethyl-0-[2,4-dichloro-5 (methylthio) phenyl] thionophosphate.

The compound is a yellowish-brown liquid (technical) which forms crystals at room temperature and below. Its molecular weight is 361.25, and its boiling point is 155°C at 0.1 torr. It is practically insoluble in water, and soluble in acetone, benzene, ethanol and other organic solvents. Chlorthiophos is a potent organophosphorus insecticide with contact and stomach poison activity. It has been found effective in sucking, biting and mining insects. Tests have been carried out world-wide, and the effective dose of the substance for specific pests is shown in Table 1 (6). Recommendations for further trials are given in Table 2. (7).

Table 1

The effective dose of chlorthiophos for specific pests

Crop	Pest	Dosage g a. i./ha or concentration (a. i.)
Pome-fruit (apple, pear)	Aphidae	0.025%
	Argyrotaenia spec.	0.025—0.0375%
	Caputa spec.	0.025%
	Carpocapsa spec.	0.025—0.0375%
	Choristoneura spec.	0.025—0.0375%
	Conotrachelus spec.	0.025%
	Epiphyas spec.	0.0375%
	Euproctis spec.	0.025%
	Grapholita spec.	0.025%
	Hyponomeuta spec.	0.025%
	Lygus spec.	0.0375%
	Malacosoma spec.	0.025%
	Pseudococcus spec.	0.0375%
	Psylla mali	0.025—0.0375%
	Quadraspidiotus spec.	0.025—0.0375%
	Rhagoletis spec.	0.025%
	Typhlocyba spec.	0.025%
Stone-fruit (cherry, peach)	Aphidae	0.025%
	Argyroploce spec. (= Cryptophlebia)	0.025—0.0375%
	Grapholita spec.	0.0375%
	Lygus spec.	0.0375%
	Rhagoletis spec.	0.025—0.0375%

Cotton	Alabama spec.	500
	Anthonomus spec.	500—750
	Aphidae	300
	Bucculatrix spec.	1000
	Diparopsis spec.	750—1000
	Earias spec.	1200—1600
	Empoasca spec.	500
	Heliothis spec. (young larvae)	1000—1200
	Pectinophora spec.	1000—1200
	Prodenia litura (= Spodoptera littoralis)	1000—1200
	Spodoptera frugiperda (= Laphygma)	750—1000
Rice	Chilo spec.	500—750
	Pachydiplosis spec.	750
	Sogatodes spec.	500—750
	Tryporyza spec.	750
Corn (Maize)	Oscinella spec.	300—600
	Ostrinia spec. (= Pyrausta)	1500
	Spodoptera frugiperda (= Laphygma)	750—1000
Potatoe	Leptinotarsa spec.	300—400
Pistachio	Agonoscena spec.	0.075%

Table 2

Recommendations for further trials

Crop	Pest	Dosage g a. i./ha or concentration (a. i.)
Pome-fruit (apple pear)	Cemiostoma spec.	0.0375%
	Hoplocampa spec.	0.025—0.0375%
	Lyonetia spec.	0.025—0.0375%
	Operophthera spec.	0.025—0.0375%
Stone-fruit	Hoplocampa spec.	0.025—0.0375%
	Operophthera spec.	0.025—0.0375%
Berries	Lygus spec.	0.0375—0.05%
Citrus	Aonidiella spec.	0.0375—0.05%
	Cacoecia spec.	0.0375—0.05%
	Trioza spec.	0.0375—0.05%

Grape vine	Typlocyba spec.	0.025—0.0375%
	Paralobesia spec.	0.025—0.0375%
	Pseudococcus spec.	0.025—0.0375%
Vegetables (beets, broccoli, beans, cabbage, cauliflower, cucumber, tomato)	Aphidae	150—250
	Atomaria spec.	300—600
	Diabrotica spec.	500—1000
	Empoasca spec.	500—750
	Epilachna spec.	500
	Etiella spec.	500—1000
	Liriomyza spec.	750—1000
	Pegomya spec.	150—250
	Pieris spec.	500
	Platynota spec.	500
	Plutella spec.	500
	Thrips spec.	150—250
Cereals	Aphidae	150—250
	Eurygaster spec.	500
Sugar cane	Aphidae	300
	Diatraea spec.	750—1000
Tobacco	Aphidae	300
	Heliothis spec.	1000—1200

Animals, housing and food

Mice (about 20 g, strain Chbi:NMRI(SPF), rats (about 200 g, strain Chbb:THOM(SPF), guinea-pigs (bastard, K. Marioth, D-6273 Waldems 1), Japanese quail (Schloß Schomberg, D-7517 Eppingen) and hens (1 year old, HNL-hybrids, Peters, D-6223 Kelkheim) were kept in groups in Makrolon^{R} cages at room temperature. Rabbits (about 4 kg, New Zealand, Vieten, D-4041 Büttgen-Vorst) were kept in individual cages at room temperature. All the animals received the standard food Altromin^{R}, Club-Korn^{R} (quail) or Deuka-Mehl^{R} (hens). Before and during the experiment, all animals were allowed free access to food and water.

METHODS

Acute oral and dermal toxicity

Acute oral toxicity studies with an observation period of two weeks were carried out on mice, rats, guinea-pigs, rabbits, hens and quails. Additional acute dermal toxicity studies were done in rats and rabbits.

Mice and rats received the substance with a metal stomach tube (by gavage), and the other animals with a (rubber) catheter of a suitable size. In the dermal test on rats, the clipped abdominal part of each test animal was brought into contact with the diluted substance in a bath (method of *Schütz* (8)). In rabbits the treated area was covered with a gauze patch (as in the patchtest of FDA (9)). A 200 cm^2 area of the back was clipped prior to application of the substance.

The LD_{50} was calculated according to *Litchfield* and *Wilcoxon* (10). For the determination of acute toxicity in rabbits and hens the calculation was done graphically.

Neurotoxicity

Two groups of ten hens each received chlorthiophos orally for one month in a dose of 2 and 10 mg/kg/day as an emulsion in water with Tween 80. A control group (7 hens) received an aqueous solution with Tween 80. The behaviour of the animals was observed daily, and walking ability was tested on a catwalk. The hens remained under observation for further four weeks after a 4-week treatment period, and were subsequently autopsied.

Eye mucosa compatibility

The procedure was as described in FDA guidelines (9): 0.1 ml amounts of a 1% aqueous solution of chlorthiophos with Tween 80 were instilled into the right conjuctival sac of three New Zealand rabbits. The left eye of each animal was not treated and served as control. In another two groups of three animals each, the treated eye was washed out with lukewarm water 2 and 4 seconds after treatment. Observations were made after 5 minutes, 1, 3 and 24 hours, and after 2, 3, 4 and 7 days.

Dermal tolerance in rabbits

Patch tests (FDA guidelines (9)) with 0.5 to 2.0% emulsions of chlorthiophos (with Tween 80) were carried out on the abraded and intact skin of the New Zealand rabbits. Groups of three rabbits per group were used. Observation period was five days.

Cholinesterase inhibition (acute tests)

All determinations of cholinesterase activity in the above described experiments with chlorthiophos were carried out using the manometric method according to *Ammon* (11). The cholinesterase activity was measured in erythrocytes, plasma and brain of rats. In all experiments acetylcholine (1×10^{-3} M) served as substrate. After oral administration of 1/2 LD_{50} dose of chlorthiophos animals were killed at given times and erythrocyte, plasma and brain cholinesterase activity was measured in groups of six animals. Erythrocyte and plasma cholinesterase activities were monitored between 10 minutes and 6 days after dosing

and brain cholinesterase activity between 10 minutes and 8 days after dosing. The manometric determinations were carried out with a respirometer according to Gilson.

Antidote experiments

The antagonistic effect of atropine and various oximes was tested *in vivo* in rats and mice intoxicated with chlorthiophos. Mice received an LD_{50} dose of chlorthiophos and rats an 1.5 LD_{50} dose of the same substance. Atropine was administered intravenously in a dose of 25 mg/kg, PAM-2 in a dose of 10 mg/kg, trimedoximebromide-4 in a dose of 5 mg/kg, obidoximechloride in a dose of 5 mg/kg, also i.v.

Separate experiments were carried out to test the possible effect of enzyme inductors on the toxicity of chlorthiophos. Two daily doses of 50 mg/kg lindane were given orally to a group of 10 mice over a period of five days. A second group of 10 mice received 25 mg/kg phenobarbital likewise twice daily over the same period. On the fifth day each of the two groups and an untreated third group (control) of 10 mice received 141 mg/kg chlorthiophos (LD_{50}) by gavage two hours after the last pretreatment with lindane or phenobarbital.

The reactivation ability of antidotes PAM-2 and trimedoximebromide was also investigated in rats poisoned with chlorthiophos. Rats were given chlorthiophos orally (6.5 mg/kg) and oximes intravenously (PAM-2 10 mg/kg, trimedoximebromide 5 mg/kg) either simultaneously or one hour after chlorthiophos.

RESULTS

Acute toxicity

The findings are shown in Table 3. Chlorthiophos was best tolerated by mice and least well by rats and rabbits. The symptoms observed were typical of organophosphorus insecticide poisoning : tremor, clonic convulsions, exophthalmos, lacrimation, salivation, difficult breathing and terminal paralysis.

Neurotoxicity in hens

In the subacute test a dose of 2 mg/kg/day chlorthiophos administered orally for one month was tolerated well. No symptoms were observed. A doze of 10 mg/kg/day was not well tolerated, the hens showed weakness, ataxia and a lack of appetite. In none of the tested hens did the typical neurotoxic symptoms of paralysis of the extremities occur.

Table 3

Acute oral and dermal toxicity of chlorthiophos in different species

Species	Application	No. of animals	Observation (days)	LD_{50} mg/kg
Mouse	p. o.	60	14	141 (109—183)
Rat	p. o.	40	14	13 (9—18)
Guinea-pig	p. o.	40	14	58 (52—65)
Rabbit	p. o.	12	14	20
Quail	p. o.	50	14	45 (37—55)
Hen	p. o.	10	30	45
Rat	dermal	60	14	58 (55—60)
Rabbit	dermal	16	14	48

All determinations were carried out with pure chlorthiophos, except dermal toxicity tests in rats and rabbits when an emulsion concentrate was used.

Calculation was dose according to *Litchfield* and *Wilcoxon* (10), except hen (p. o.) and rabbit (p. o. and derm.): the toxicities were determined graphically on a probability net-work (Schleicher & Schuell, No. 297 1/2).

Eye mucosa compatibility

The 1% aqueous emulsion of chlorthiophos applied to the eye of the rabbit was well tolerated .

Dermal tolerance

Aqueous emulsions of 0.5, 1.0 and 2.0% chlorthiophos were well tolerated by the intact clipped skin of the rabbit. Scarified skin showed minimal signs of intolerance to 0.5 and 1.0% emulsions, and signs of moderate intolerance to the 2.0% emulsion.

Cholinesterase inhibition

The maximum cholinesterase inhibition in the blood of the rat after oral administration of 6.5 mg/kg chlorthiophos (0.5 x LD_{50}) was reached between 2 and 6 hours. The inhibition was 68% in erythrocytes and 89% in the plasma (Figs. 1 and 2). A complete return to normal was observed after four days. After administration of the same dose of chlorthiophos the maximum cholinesterase inhibition in the brain of the rats was found after 6 hours (Fig. 3). The inhibition amounted to 75%. No inhibition of brain cholinesterase was found up to 30 minutes after oral administration of chlorthiophos. A spontaneous reactivation of cholinesterase was observed from the 4th day after administration onwards. After four days the degree of inhibition was 52%, after five days 32% and after eight days brain cholinesterase activity was normal.

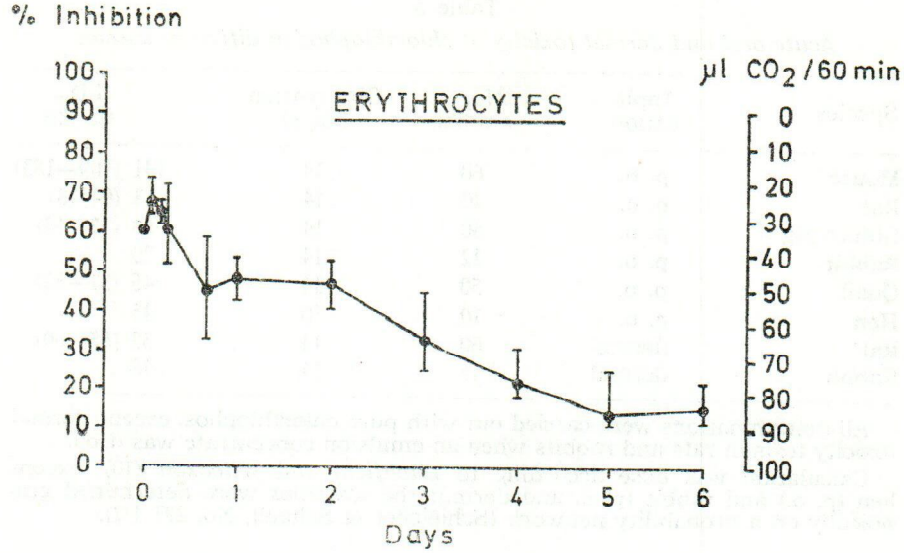

Fig. 1. *Time-effect study of cholinesterase inhibition in rats' erythrocytes from 10 minutes to 6 days after oral administration of 0.5 × LD_{50} of chlorthiophos*

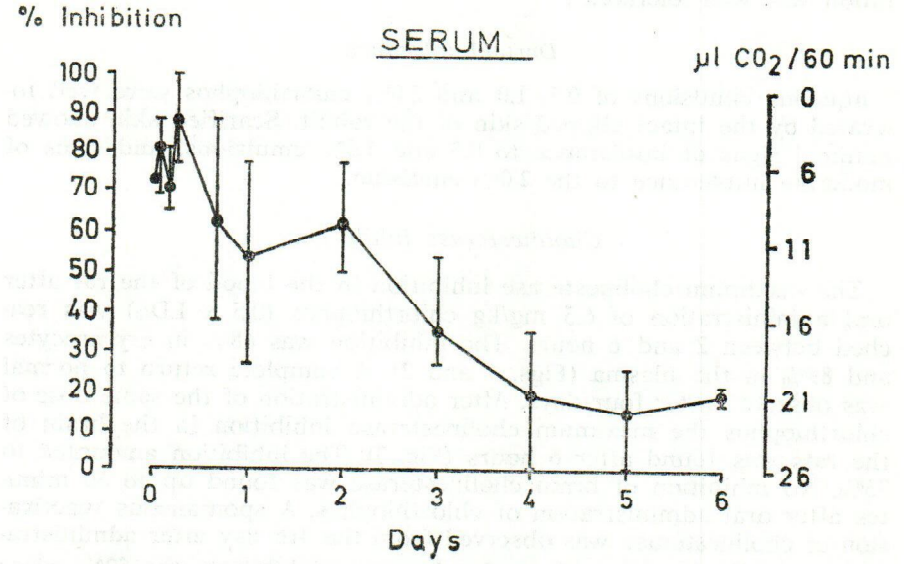

Fig. 2. *Time-effect study of cholinesterase inhibition in rats' plasma from 10 minutes to 6 days after oral administration of 0.5 × LD_{50} of chlorthiophos*

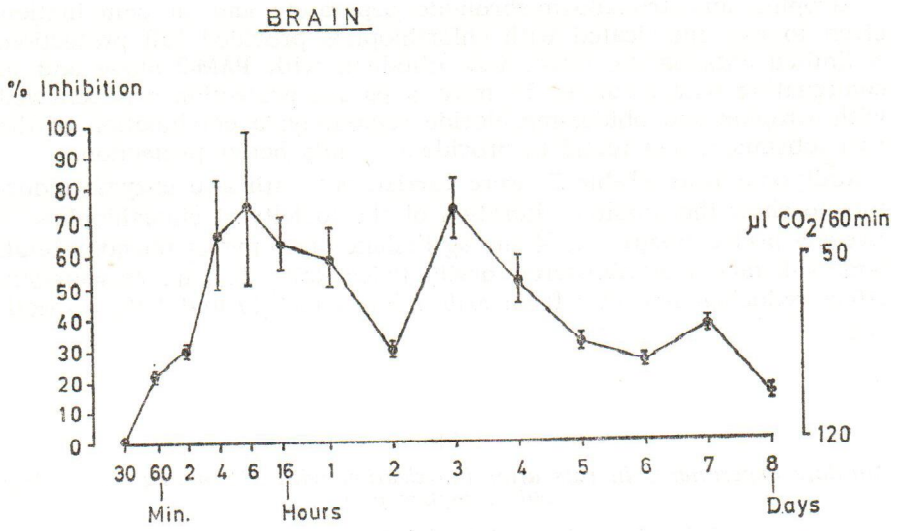

Fig. 3. *Time-effect study of cholinesterase inhibition in rats' brain from 30 minutes to 8 days after oral administration of 0.5 × LD_{50} of chlorthiophos*

Cholinesterase reactivation in rat blood with antidotes

The results are summarized in Table 4. Obidoximechloride showed a pronounced reactivating effect on erythrocyte and plasma cholinesterase. No effect on plasma cholinesterase was observed with PAM-2. With erythrocyte cholinesterase, however, favorable results were obtained only if PAM-2 was given to rats one hour after intoxication.

Table 4

Cholinesterase inhibition in erythrocytes and plasma of rat after oral administration of 6.5 mg/kg chlorthiophos and intravenous aplication of oximes

Antidote	Time of antidote administration	Chlorthiophos – inhibition of cholinesterase (%) in:		Chlorthiophos + antidote — inhibition of cholinesterase (%) in:		Reactivation (%) in:	
		Erythrocytes	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma
Obidoxime (5 mg/kg)	simultaneous	64	66	45	31	30	53
	1 hour later	57	77	39	54	32	30
PAM-2 (10 mg/kg)	simultaneous	62	85	54	81	13	5
	1 hour later	62	77	37	65	40	16

Atropine and trimedoximebromide separately and in combination given to rats intoxicated with chlorthiophos provided full protection. A limited antagonistic effect was obtained with PAM-2 alone and in combination with atropine. In mice, a partial protection was achieved with atropine and obidoximechloride separately; a combination of the two substances was found to provide a slightly better protection.

Additional tests (Table 7) were carried out with two enzyme inductors to show the possible alteration of the toxicity of chlorthiophos. A five-day pretreatment wih 50 mg/kg lindane or 25 mg/kg phenobarbital, both substances administered orally twice daily, had an antagonistic effect reducing mortality from 5/10 animals to 0/10 and 1/10, respectively.

Table 5

Antidote experiments in rats after intoxication with 19.5 mg/kg ($1.5 \times LD_{50}$) chlorthiophos p. o.

Antidote (i. v.)	Mortality
Atropine (25 mg/kg)	0/10
Trimedoxime (5 mg/kg)	0/10
Atropine (25 mg/kg) + trimedoxime (5 mg/kg)	1/10
PAM-2 (10 mg/kg)	2/10
Atropine 25 mg/kg + PAM-2 10 mg/kg	5/10
None	8/10

Table 6

Antidote experiments in mice after intoxication with 141 mg/kg ($1 \times LD_{50}$) chlorthiophos p. o.

Antidote (i. v.)	Mortality
Atropine (25 mg/kg)	3/10
Obidoxime (5 mg/kg)	2/10
Atropine (25 mg/kg) + obidoxime (5 mg/kg)	1/10
None	8/10

Table 7

Reduction of acute toxicity of chlorthiophos in mice after a 5-day pretreatment with lindane or phenobarbital

Substance	Dose in mg/kg	Mortality
Chlorthiophos	141	5/10
Lindane + chlorthiophos	2 × 50/day 141	0/10
Phenobarbital + chlorthiophos	2 × 25/day 141	1/10

DISCUSSION

In earlier studies we dealt with two phosphoric acid esters bromophos and bromophos-ethyl, both of which contain dichlorbromphenol as the acidic group. Bromophos is a dimethyl phosphoric acid ester, and bromophos-ethyl is its diethyl homologue. Chlorthiophos is another related substance which contains a methylthio group instead of bromide in the phenol part of the bromophos-ethyl molecule. The substitution of the two methyl groups in bromophos for two ethyl groups in bromophos-ethyl considerably increased the toxicity, which was further noticeably increased in chlorthiophos. The reactivation of cholinesterase with suitable oximes (obidoximechloride, trimedoximebromide) and the antidote effect of atropine applied alone and in combination with the oximes were good, and could be compared with the previous results (3) with bromophos-ethyl. The application of atropine-oxime combination in the case of poisoning is therefore reasonable. Contrary to this with the primarily far less toxic dimethyl analogue, bromophos, atropine and oximes have hardly any effect on intoxication. The fact that the pretreatment with some enzyme inductors (lindane, phenobarbital) reduces the toxicity may be connected with their effect on the microsomal drug-metabolizing enzymes of the liver. The detoxification effect of lindane or barbiturates is certainly of no practical importance for antidotal application.

Sažetak

AKUTNA TOKSIČNOST I INHIBICIJA HOLINESTERAZE CHLORTHIOPHOSOM IN VIVO

Opisana je akutna toksičnost insekticida klortiofosa, čija je glavna komponenta 0,0-dietil-0-[2,5-dikloro-4-(metiltio)fenil] tionofosfat u kombinaciji sa 0,0-dietil-0-[4,5-dikloro-2-(metiltio)fenil]tionofosfatom i 0,0-dietil-0-[2,4-diklor-4-(metiltio)fenil] tionofosfatom. Peroralne LD_{50} vrijednosti su: štakor 13

mg/kg, miš 141 mg/kg, zamorče 58 mg/kg, kunić 20 mg/kg, prepelica 45 mg/kg, kokoš 45 mg/kg. Dermalna LD_{50} za kunića je 48 mg/kg a za štakora 58 mg/kg.. Neurotoksičnost na kokošima nije bila primjećena. Maksimalna inhibicija holinesteraze u eritrocitima štakora je nađena nakon dva sata a u plazmi nakon četiri sata. U mozgu štakora maksimalna inhibicija holinesteraze nađena je nakon šest sati. Oporavak holinesterazne aktivnosti opažen je u krvi štakora nakon četiri a u mozgu nakon osam dana. Lindan i fenobarbital smanjuju akutnu toksičnost klortiofosa, ako se davaju dvaput dnevno pet dana prije insekticida. Atropin i neki oksimi (obidoksim i trimedoksim) mogu antagonizirati toksične učinke klortiofosa.

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