Effect of Chloridazone on the Animal Organism

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Abstract. The acute toxic effect of the herbicide chloridazone and mitochondrial respiration were investigated and typical clinical signs of intoxication were described in rats (Wistar), pheasants (*Phasianus colchicus*) and sheep (Slovak Merino). The LD_{50} of chloridazone was calculated to be for rats 800 mg/kg bw (range 552 to 1160 mg/kg bw) and for pheasants 3684 mg/kg bw (range 1768 to 7677 mg/kg bw). According to WHO chloridazone is moderately toxic for rats and slightly toxic for pheasants. The LD_{50} for sheep is 161 mg/kg bw (range 76 to 340 mg/kg bw). Chloridazone thus presents an acute risk for ruminants, which is in coincidence with the WHO classification characterising it as a very toxic compound. The following clinical features of intoxication were observed after p.o. administration of chloridazone: apathy, dyspnoea, hyperventilation, hypersalivation (sheep – foam hypersalivation), paralysis, tonic-clonic convulsions and death in clonic convulsions. Very quick *rigor mortis.*

Chloridazone interfered with mitochondrial respiration in the liver of rats yet its mode of action was different from that of succinate substrate or glutamate-malate. Succinate dependent respiration was significantly decreased in both states (3 and 4) of respiration. Glutamate-malate respiration was not changed in state 4, though it significantly increased in state 3 after ADP administration. RCP (respiration control proportion) value was increased on using either of the substances.

Key words: Chloridazone — Clinical features — LD_{50} — Mitochondrial respiration — Animals

Introduction

In most industrial countries, drugs (including veterinary medicines), food additives, industrial chemicals and pesticides, to which human and other living organisms may be exposed in the environment, have to be tested for toxicity. Initial toxicity studies would usually be carried out to determine the approximate range of toxic doses.

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Acute toxicity tests are those designed to determine the effects which occur within a short period after dosing. These tests can determine a dose-response relationship and the LD_{50} value (Timbrell 1989).

This article presents a toxicological study of chloridazone on some species of animals. Chloridazone (pyramin or pyrazone) is the best known pyridazone herbicide used for the control of weeds in beet (El-Abyad et al. 1991).

Three ways of effect have been distinguished as far as pyridazone herbicides are concerned: photosynthesis inhibition, carotenoid biosynthesis inhibition and their involvement in lipid biosynthesis (Fedtke 1982). The main effect of herbicidal preparations on the basis of chloridazone is the inhibition of the Hill photosynthetic reaction in plants. Since the latter photosynthetic reaction is not present in the animal organism, it is important to explain the effect of these preparations in animals.

According to literary data, the substances which interfere with energy metabolism in animals usually influence photosynthetic reactions in plants. Photosynthesis and the reactions of oxidative phosphorylation could be explained on the basis of the chemiosmotic theory according to Hinkle and McCarty (1978).

According to toxicological studies on animals chloridazone has a weakly or moderately irritant effect on the eye and skin. No adverse effects were observed on reproduction parameters, and no signs of teratogenic effects were noted. Chloridazone can be assessed as not mutagenic and there were no signs of an oncogenic response (Registration Department BASF Japan Ltd. 1992).

The aim of this study was to determine LD_{50} for the rat, pheasant and sheep, to describe common clinical features in acute exposure and to study the parameters of mitochondrial energy production in the liver of rats.

Materials and Methods

The tests were accomplished according to the OECD methods No 401 (1987) and No 205 (1984) Chloridazone LD_{50} values were determined by the use of the method of interpolation according to Roth et al (1962) This method enables fast economical and precise LD_{50} determination with the upper and lower level of reliability Advantage of this method is the fact that we can use a smaller amount of experimental animals with the assumption of high exactness of results

For determination of LD_{50} were used three kinds of animals Twenty male Wistar rats weighing 50–60 g, 5–6 months old, which were divided into two experimental groups (experimental group I – dose 800 mg/kg bw, II – dose 1000 mg/kg bw) and control group Twenty four pheasants (*Phasianus colchicus*) (12 cock and 12 chicken) weighing 1300 1500 g, which were divided also into two experimental groups (with doses I – 2000 mg/kg bw, II – 5000 mg/kg bw) and control group In the last determination we used four Slovak Merino sheep weighing 19–22 kg, divided into experimental group I (dose 130 mg/kg bw) and II (dose 200 mg/kg bw) and control group Chloridazone diluted in water was administered to each experimental animal p o through a gastric tube

To study mitochondrial respiration, the surviving rats (dose 800 mg/kg bw) were sacrificed and liver mitochondria were isolated according to Johnson and Lardy (1967) The isolated mitochondria were used to study oxidation processes in the above mentioned organs, using a Clark-type oxygen electrode of the WTW Co

We measured the oxygen consumptıon ın state 4 (ın the absence of ADP – adenosınediphosphate), ın state 3 (state of actıvated respıratıon after addıtıon of ADP) and after addıtıon of 2,4 – dınıtrophenol (DNP) wıth the use of succınate substrate or glutamate-malate The values of the respıratory control ratıo (RCR) were calculated from the proportıon of oxygen consumptıon ın state 3 and 4 The content of proteın ın ısolated mıtochondrıa was estımated by the method of Hartree (1972)

The effect of chlorıdazone ın *ın vıtro* condıtıons (doses 100, 200, 250 and 300 μg/mg of proteıns – doses were calculated from doses admınıstered ın *ın vıvo* experıments) on the respıratıon of lıver mıtochondrıa ın rats was observed

The results were statıstıcally evaluated accordıng to Student's t-test

Results

According to WHO (1975), chloridazone is moderately toxic for rats and slightly toxic for pheasants For ruminants chloridazone presents an acute risk, which coıncides with the WHO classification, characterising it as a very toxic compound (Table 1)

Table 1. Chlorıdazone LD_{50} value ın rats, pheasants and sheep

Specıes of anımal	LD 50 (mg/kg bw)	Lower level of relıabılıty (mg/kg bw)	Upper level of relıabılıty (mg/kg bw)
rat	800 0	551 5	1160 4
pheasant	3684 0	1768 0	7677 0
sheep	161 2	76 5	340 0

The following clinical symptoms of ıntoxication were observed after p o. admınıstratıon of chloridazone· apathy, hyperventilation, dyspnoea, hypersalıvation (sheep-foam hypersalivation), paralysıs, tonic-clonıc convulsions and death in clonic convulsions. Very quick *rıgor mortıs.*

Succinate dependent respiratıon decreased significantly from 8.67 to 3.62 mmol/min.kg_{prot} in state 4 and from 16.91 to 11.83 mmol/min.kg_{prot} in state 3. RCP value increased significantly from 2.00 to 3.32 (Table 2).

Table 2. Lıver mıtochondrıal respıratıon on succınate substrate – *ın vıvo* experıment

Succınate	Control group $x \pm s_x$ (mmol/mın kg_{prot})	Experımental group $x \pm s_x$ (mmol/mın kg_{prot})
State 4	8 67 ± 1 00	3 62 ± 0 26*
State 3	16 91 ± 1 30	11 83 ± 0 80*
RCP	2 00 ± 0 17	3 32 ± 0 24*

*$p < 0\ 05$ (agaınst control group), mmol/mın kg of mıtochondrıal proteıns

Glutamate – malate dependent respiration decreased in succinate dependent respiration from 2.71 to 2.49 mmol/min.kg_{prot} in state 4, but it increased significantly from 4.87 to 8.98 mmol/min.kg_{prot} in state 3. RCP increased significantly from 1.86 to 3 67 (Table 3)

Table 3. Liver mitochondrial respiration on glutamate-malate substrate – *in vivo* experiment

Glutamate-Malate	Control group $x \pm s_x$ (mmol/min kg_{prot})	Experimental group $x \pm s_x$ (mmol/min kg_{prot})
State 4	2 71 ± 0 27	2 49 ± 0 18
State 3	4 87 ± 0 20	8 98 ± 0 84*
RCP	1 86 ± 0 21	3 67 ± 0 36*

*$p < 0\ 05$ (against control group), mmol/min kg of mitochondrial proteins

Our results obtained in the *in vitro* experiments confirmed the results from *in vivo* experiment.

In state 4, succinate inhibition was recorded in dependency on chloridazone concentration. It decreased from 8.7 to 2.2 mmol/min.kg_{prot} in state 4 and from 14.4 to 4.9 mmol/min.kg_{prot} in state 3. Glutamate-malate inhibition was recorded from 2 9 to 1.4 mmol/min.kg_{prot} in state 4 and from 4.9 to 2.1 mmol/min.kg_{prot} in state 3 (Table 4).

Table 4. Liver mitochondrial respiration – *in vitro* experiment

Substrate	State	Control group	100 (μg/mg prot)	200 (μg/mg prot)	250 (μg/mg prot)	300 (μg/mg prot)
Succinate	4	8 7	4 4	3 6	2 1	2 2
	3	14 4	13 2	9 4	7 7	4 9
Glutamate	4	2 9	2 3	1 9	1 4	1 4
Malate	3	4 9	6 6	5 1	4 2	2 1

μg of chloridazone/mg of mitochondrial proteins

Discussion

All chemicals can be classified according to LD_{50}. The LD_{50} value may vary for the same compound between different animal species. In our work we determined LD_{50} for rats (rodent), wildlife bird and sheep (mammal – ruminant). These groups of animals are anatomically and physiologically different, therefore also the LD_{50} values of chloridazone vary (Table 1).

The herbicide chloridazone has an adverse effect on the animal organism provoking obvious clinical signs in the LD_{50} dose. The mechanism of chloridazone action has not yet been fully elucidated.

Chloridazone (pyrazone) and bentazone belong to the group of pyridazone herbicides (Cremlyn 1985). The herbicidal effect of pyridazone compounds on chloridazone base is exerted by inhibition of the Hill photosynthetic reaction in plants. In the animal organism this reaction does however not exist, yet these compounds may interfere with the energy metabolism in the animal organism. Chloridazone can be assumed to interfere with the energy metabolism as the described clinical signs and rapid onset of *rigor mortis* are very similar to the effects of bentazone (Neuschl et al. 1993), which was shown to interfere with the energy metabolism in the animal organism. The results of mitochondrial respiration in the liver of rats also support this assumption.

Interference of chloridazone with mitochondrial respiration in the liver of rats is different on using succinate substrate or glutamate-malate substrate. Succinate dependent respiration was significantly decreased in both states of respiration *in vivo*. Glutamate-malate respiration was however not changed in state 4, yet it was significantly increased in state 3 after the ADP administration. The RCP value was significantly increased on using either substrate. Chloridazone application *in vitro* in dependency on its concentration confirmed the results obtained in experiments *in vivo*. Succinate dependent inhibition of respiration was recorded in both states (3 and 4), while on using glutamate-malate substrate in state 3, respiration increased in the beginning of the experiment. On increasing the concentration of chloridazone, inhibition of respiration was recorded.

Mitochondrial respiration was inhibited by chloridazone in state 4 (i.e. "control state") on FAD (flavin-adenine dinucleotide) as well as NAD (nicotinamide-adenine dinucleotide)-dependent substrate. After the evoked exogenous phosphorylation (after ADP addition), chloridazone reacted differently on each substrate.

Chloridazone can be assumed to interfere with energy production by influencing succinate dehydrogenase. This contention is supported by our results of the *in vitro* experiment.

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References

Cremlyn R (1985) Pesticides, SNTL, Praha (in Czech)

El-Abyad M S , Gabr M A , Abu-Taleb Amira M (1991) Metabolic activities of sugar-beet pathogens *Fusarium solani* (Mart) Sacc and *Sclerotium rolfsii* Sacc under pyramin stress Plant and Soil **132,** 103—112

Fedtke C (1982) Biochemistry and Physiology of Herbicide Action p 202, Springer-Verlag Berlin, Heildelberg, New York, Germany

Hartree E F (1972) Determination of protein A modification of the Lowry method that gives a linear photometric response Anal Biochem **48,** 422—427

Hinkle P C , McCarty R E (1978) Energy conservation in photosynthesis and respiration, In Biochemistry and Physiology of Herbicide Action, (Ed C Fedtke) pp 114—115 Springer-Verlag Berlin Heildelberg, New York

Johnson D , Lardy H (1967) Isolation of liver mitochondria, In Methods in Enzymatology, Vol 10, Colowick and Kaplan, pp 94—96 Academic Press, New York and London

Neuschl J , Kačmár P , Legáth J , Tomáš J (1993) The effect of the Czechoslovak developmental herbicide bentazone on some parameters of sheep health under the conditions of subchronic intoxication, Vet Med **37,** pp 161—167

OECD Guideline for testing of chemicals 205 (1984) Avian Dietary Toxicity Test

OECD Guideline for testing of chemicals 401 (1987) Acute Oral Toxicity

Registration Department, Agricultural Chemical Division, BASF Japan Ltd (1992) Summaries of toxicity studies on chloridazon J Pesticide Sci **17,** 171—176

Roth Z , Josífko M , Malý V , Trčka V (1962) Statistical Methods in Experimental Medicine, p 297, SZN, Praha (in Czech)

Timbrell J A (1989) Toxicity testing and risk assessment, In Introduction to Toxicology, Taylor and Francis, pp 129—131, Academic Press, London, New York, Philadelphia

WHO Chronicle (1975) Recommended classification of pesticides by hazard, pp 397–401