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CROWN-ETHERS TOXICITY PARAMETERS AND THEIR CUMULATIVE PROPERTIES IN SHORT-TERM EXPERIMENTS

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The present article illustrates the experimental results of acute and sub-acute toxicity of crown-ethers (12-crown-4, 15-crown-5, and 18-crown-6). The experiments were performed upon biological objects of different complexity of structure-functional organization. Crown-ethers impaired growth and reproduction of *Daphnia* and unicellular Algae at 5 and 10 mg/l concentrations and caused toxic action upon tissue cellular culture (*J₉₂₉*, *X-63*, *Hep-2*, *Vero*) and buccal epithelium at 0.05 and 0.5 mg/l concentrations. Cytotoxic action of the investigated xenobiotics was connected with DNA, RNA, and protein biosynthesis disturbances. The results of experiments with rats, mice, and guinea-pigs showed crown-ethers to be moderately toxic and extremely cumulative substances. The cellular cultures and buccal epithelium could be used in express-evaluation of biological activity of newly synthesized xenobiotics, as the reliable indexes of determination of potential danger of chemical compounds for warm-blooded animals.

Key words: crown-ethers, toxicity parameters, cumulative properties, short-term experiments

Параметри токсичності та кумулятивні властивості краун-ефірів у короткострокових експериментах. Кратенко Р.І. – Надано результати експрес-оцінки гострої біологічної активності групи краун-ефірів (12-краун-4, 15-краун-5, 18-краун-6), здійсненої на біологічних об'єктах різної складності структурно-функціональної організації. Краун-ефіри порушували ріст і розмноження дафній та одноклітинних водоростей у концентраціях 5 та 10 мг/л, справляли токсичну дію на тканинні культури клітин (*J₉₂₉*, *X-63*, *Hep-2*, *Vero*) і буккальний епітелій у концентраціях 0,05 та 0,5 мг/л відповідно. Цитотоксична дія дослідних сполук була пов'язана з порушенням біосинтезу ДНК, РНК та білку. Експерименти на щурах, мишах та морських свинках виявили, що краун-ефіри відносяться до помірно токсичних та надзвичайно кумулятивних сполук. Культури клітин і буккальний епітелій можуть бути використані для експрес-оцінки біологічної активності хімічних речовин, як надійні показники їх потенційної небезпечності для організму теплокровних тварин.

Ключові слова: краун-ефіри, параметри токсичності, кумулятивні властивості, короткострокові експерименти.

INTRODUCTION

Within the last 20-30 years, the volume and assortment of compounds produced by organic synthesis industry has been significantly widening. In technically developed countries the production of macroheterocyclic crown-ethers has been particularly increased. These compounds possess such unique properties as solubility in many non-aqueous mediums, high stability, selectivity of oxido-

reductive reactions, ability to form complexes with metals, etc. Therefore, crown-ethers find growing application in electrochemistry, pharmacy, medicine. It has been estimated that only Russian chemistry industry produces more than 10,000 tons of macroheterocyclic compounds annually. To be noted, one ton of the produce form about 40 m³ of waste water. The latter may cause serious problems reaching natural water objects in terms of creating significant obstacles in adequate water delivery to population and in disturbing the natural self-cleansing processes of ponds and rivers.

The essential problem of biology and practical toxicology encompasses determination ways of chemical compounds various parameters, i.e. general biological activity, toxicity class, cumulative, skin-resorbative, organoleptic properties. Newly-synthesized xenobiotics testing parameters should be of the utmost importance.

MATERIALS AND METHODS

The express-evaluation of crown-ether group (12-crown-4, 15-crown-5, 18-crown-6) biological activity was performed using biological objects of different complicity degree – from mono-cellular to organism levels. Determination of crown-ethers toxicity degree was carried out using a representative of lower crustacean – *Daphnia magna*, monocellular Algae (*Dunaliella salina*, *Pedinomonas tenius*), tissue cultures (*J₉₂₉*, *X-63*, *Hep-2*, *Vero*) and native cells of buccal epithelium. The substances influence on biological objects was studied according to the following indexes: growth, multiplication and survival of *Daphnia*; disturbance of Algae mobility, their adhesion and coagulation; loss of cellular cultures ability to uptake neutral red colorant, appearance of cells pathologic forms, cells adhesion; decrease in buccal epithelium cells electronegativity [1]. The experimental concentrations of crown-ethers were 1.0, 5.0, 10.0, 20.0, 30.0, 50.0 mg/l.

Determination of crown-ethers toxicity parameters in short-term experiments was performed using warm-blooded animals – Vistar population rats (180-210 g), white mice (21-23 g), guinea-pigs (350-380 g) [2]. The doses of crown-ethers were chosen so that the determination of lethal effect could be performed in doses interval of LD₀-LD₁₀₀. The substances were administered directly in stomach in pure form with the usage of metal catheter one time, with the following observation of the animal within 15 day. We registered the time of animal's death and the total sum quantity of administered substance. The dead and survived animals were subjected to path-morphologic autopsy.

Chronic poisoning is known to be closely related to cumulation of the poison itself in the organism or/and to the alterations evoked by the poison. Cumulation of crown-ethers in the animals organism was investigated according to Lim's method [3].

RESULTS AND THEIR DISCUSSION

The preliminary evaluation of crown-ethers biological activity using *Daphnia* showed all investigated compounds in concentrations 5 mg/l and higher to be slowing down growth and multiplications of the lower crustacean. In concentration of 10 mg/l

the substances evoked sterility effect which was characterized by the absence of young animals offsprings. Crown-ether concentrations higher than 20 mg/l resulted in 100 % death of young and mature *Daphnia*. The threshold concentration of the crown-ethers group according to their influence on *Daphnia magna* was established at the level of 5 mg/l.

The unicellular Algae *Dunaliella salina*, *Pedinomonas tenius* were proven to be somewhat less sensitive to the action of the investigated compounds. In concentrations of higher than 10 mg/l crown-ethers action decreased their mobility, resulted in their adhesion and subsequently in their coagulation precipitation to the bottom of model ponds. The threshold concentration of the crown-ethers group according to their influence on unicellular Algae was established at the level of 10 mg/l.

The investigation of crown-ethers toxicity extent using cellular cultures (*J₉₂₉*, *X-63*, *Hep-2*, *Vero*) displayed the investigated compounds in concentrations higher than 1 mg/l to decrease the ability of cells to sprawl and uptake neutral red colorant as well as to result in cells pathologic forms appearance, cells corrugation and their slipping form glass.

The investigation of ³H-thymidine, ³H-uridine and ¹⁴C-leucine incorporation in the cellular cultures manifested the significant inhibition of these processes, which signified the reduction of DNA, RNA and protein biosyntheses at the influence of crown ethers. The sensitivity of liver (*Hep-2*) and green monkey kidney (*Vero*) cells cultures was somewhat higher than sensitivity of other cultures. Non-action concentrations on cellular cultures were 0.05 mg/l for 12-crown-4, 0.2 mg/l for 15-crown-5 and 0.05 mg/l for 18-crown-6.

Determination of crown-ethers biological activity in relevance to buccal epithelium cells showed their high sensitivity to the toxic agents. The crown-ethers concentrations of 1 mg/l and higher decreased electro-kinetic properties of buccal epithelium nuclei in 30 % and more. Threshold concentration was established at the level of 0.5 mg/l; maximal non-action one – 0.1 mg/l. The investigated compounds influence on buccal epithelium cells nuclei electronegativity allows to conclude membranotropic action of crown-ethers at the stage of preliminary evaluation.

The results of express-evaluation of crown-ethers acute toxicity signified the higher sensitivity of buccal epithelium cells to the investigated substances compared to other models.

The primary task of toxicological investigations using warm-blooded animals should be establishment of toxicity parameters clinical pattern of poisoning, species and gender sensitivity due to substances per oral way of administration in the organism of rats, mice and guinea pigs. Crown-ethers are evaluated to be moderately toxic compounds (3^d class of toxicity), which do not possess species and gender sensitivity (tab.1).

The average time of animals death was in the interval of the first half of day. The clinical pattern of acute poisoning was characterized by CNS disturbance symptoms (tremor, seizures, absence of reactions upon sound and pain stimuli),

homodynamic and respiration impairment. Within 1-5 minutes after crown-ether administration the animals developed shiver, increased heartbeat, increased respiration rate, movements coordination disturbance, skin and mucous membranes cyanosis. Depending upon substances doses administered to animals, colonic seizures ending up with the animal death arose in time interval of 30 min – 14 h 50 min.

Table 1

Crown-ethers toxicity parameters

Animal species	Substance	Toxicity parameters, g/kg		ET ₅₀ , h-min	Kc
		LD ₅₀	LD ₁₀₀		
White rats	12-crown-4	1.17	3.0	1-50	0.312
White mice		1.26	3.0	1-28	
Guinea pigs		1.30	3.0	1-30	
White rats	15-crown-5	1.35	3.5	14-20	0.71
White mice		1.46	3.5	12-40	
Guinea pigs		1.40	3.5	14-50	
White rats	18-crown-6	1.27	3.0	0-42	0.54
White mice		1.23	3.0	0-30	
Guinea pigs		1.30	3.0	0-35	

The alteration of inner organs was characterized by plethora and dystrophic changes in liver, kidney, heart, brain and lymphoid apparatus.

The investigation of crown-ethers ability to be cumulated in the organism or/and to cumulate action effects allowed us to establish coefficients of cumulation – 0.312, 0.71, 0.54 for 12-crown-4, 15-crown-5, 18-crown-6 correspondently. According to the classification of cumulation degree all the substances belong to extremely cumulative compounds. The most toxic and cumulative substance is 12-crown-4, the least toxic and cumulative one – 15-crown-5.

The evaluation of the macrocyclic compounds influence upon skin and mucous teguments established the substances to possess weak skin-irritating and skin-resorbitive action. The experimental substances penetration effect through the intact skin was investigated by blood biochemiluminescent method. The results showed

blood biochemiluminescence of experimental animals to grow increased from the first hour of the chemical compounds application whereas clinical biochemical methods allowed to determine the compounds ability to penetrate through skin in the remote periods of observation (on 20-30 days). This fact gave the opportunity to use the blood biochemiluminescent method for the express-evaluation of the experimental substances penetration through the intact skin.

CONCLUSIONS

1. Acting on the different levels of structure-functional organization of biological objects, crown-ethers are able to disturb growth and reproduction of *Daphnia* and unicellular Algae, to cause the toxic action upon the tissue cells cultures, buccal epithelium and organism of warm-blooded animals. The most sensitive bio-objects to the action of crown-ethers were the tissue cells cultures and buccal epithelium. The cytostatic effect of crown-ethers was based upon disturbance of DNA, RNA and protein biosynthetic processes.

2. The crown-ethers threshold concentrations by the compounds action upon *Daphnia* and unicellular Algae (*Dunaliella salina*, *Pedinomonas tenius*), tissue cellular cultures (*J₉₂₉*, *X-63*, *Hep-2*, *Vero*), buccal epithelium are established at the levels of 5.0, 10.0, 0.05, and 0.5 mg/l correspondently.

3. The parameters of the experimental compounds acute toxicity displayed crown-ethers to be moderately toxic and extremely cumulative xenobiotics which do not possess species and gender sensitivity.

4. Crown-ethers are able to penetrate through the intact warm-blooded animal skin and possess skin-irritating and skin-resorbitive properties.

5. Blood biochemiluminescent method could be used for the express-evaluation of the experimental substances penetration through the intact warm-blooded animal skin.

6. The tissue cellular cultures of human liver (*Hep-2*), green monkey kidney (*Vero*) and of buccal epithelium might be used in express-evaluation of biological activity of newly synthesized xenobiotics, as the reliable indexes of determination of potential danger of chemical compounds for warm-blooded animals.

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Параметры токсичности и кумулятивные свойства краун-эфиров в краткосрочных экспериментах. Кратенко Р.И. – Приведены результаты экспресс-оценки острой биологической активности группы краун-эфиров (12-краун-4, 15-краун-5, 18-краун-6), осуществленной на биологических объектах различной сложности структурно-функциональной организации. Краун-эфиры нарушали рост, размножение дафний и одноклеточных водорослей в концентрациях 5 и 10 мг/л, оказывали токсическое действие на тканевые культуры клеток (*J₉₂₉*, *X-63*, *Hep-2*, *Vero*) и буккальный эпителий в концентрациях 0,05 и 0,5 мг/л соответственно. Цитотоксическое действие исследуемых соединений было связано с нарушением синтеза ДНК, РНК и белка. Эксперименты на крысах и морских свинках показали, что краун-эфиры относятся к умеренно токсичным и чрезвычайно кумулятивным веществам. Культуры клеток и буккальный эпителий могут быть использованы для экспресс-оценки биологической активности химических соединений, как надежные показатели потенциальной их опасности для организма теплокровных животных.

Ключевые слова: краун-эфиры, параметры токсичности, кумулятивные свойства, краткосрочные эксперименты.