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MATERIALS FOR HUMAN HEALTH RISK ASSESSMENT OF TETRACYCLINE INTAKE WITH FOOD

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INTRODUCTION

Tetracyclines are antibacterial drugs with broad e ffect and combine a group of structurally close substituted polyoxyethylene-polycarbonyl hydroaromatic compounds produced by bacteria of the Streptomyces genus. Chemical modification of these substances produce semisynthetic tetracycline [1,2]. Basic compounds of te tracycline were obtained in 1948-1952. The representatives of this family are characte rized by a common spectrum and mechanism of antimicrobial action, full of cross-resistance and similar pharmacological characteristics. Differences are related to the level of antibacterial effect, absorption characteristics, distribution , metabolic basis in organisms, acceptability, and some physical and chemical properties [3]. Thirty-five compounds are described in this group.

Tetracyclines by their mechanism of biological action are related to a group of antibiotics inhibiting protein synthesis. In bacterial cells, tetracycline is an inhibitor of the protein chain elongation, and prevents binding of the next aa-t of RNA.

Tetracycline antibiotics are used in the treatment an d prevention of bacterial infections of both human and animals. In animal husbandry, they ar e used as a therapeutic and veterinary drugs, growth stimulant [4].

The use of tetracycline as a growth stimulant in livestock leads to increase in average of 4-5% body weight and feed consumption per unit of growth is reduced by 5-8%, activates body's resistance and cuts period of sagination. Addi ng these antibiotics to the diet of birds has a stimulating effect on its growth, egg production, hatching egg quality; contributes to a more efficient use of feed and better absorption of protein [5].

In terms of food hygiene main danger of residual antibiotics for human is that through the feed along with agricultural products they get into human body, where it has negative impact on gastro-intestinal microflora and lower resistance to infections and contribute to the growth of antibiotic resistance [4].

There are significant differences in standardization of tetracycline residues (MRLs ≤ 0,1-1,2 mg/kg) in the legislation of the Eu ropean Union, the US, norms and standards of the Codex Alimentarius, which are significantl y different from the requirements of the Customs Union regulatory documents (≤ 0,01 mg/kg of product). So MRL in milk under the Codex Alimentarius and the EU legislation is standardized at no more than 100 mg/kg; the US does not regulate and countries of the Eurasian Customs Union (CU) established zero tolerance.

This study is done in accordance with the Working Principles for Risk Analysis for Food Safety for Application by Governments of Codex Alimentarius Commission (CAC/GL 62-2007) of [45].

I. HAZARD IDENTIFICATION

Hazard identification of tetracycline residues was done on the basis of the available scientific literature results of different researches, including tests on human.

Tetracycline antibiotics are more than 52% of all antibiotics used in animal husbandry and veterinary medicine in Europe. [6] Tetracyclines in therapeutic doses have bacteriostatic effect.

Semisynthetic tetracycline based on oxy- and chlortetracycline and carboxamide derivatives of tetracycline with prolonged effect are used in livestock. In the first hours of injection, it has a number of differentiating properties, which includes high solubility in water with different pH In terms of fo.2.4(l)-6.3T[1,2].s

pertussis, the majority of Enterobacteriaceae: Escherichia coli, Enterobacter spp., including

The reason for revising the results of the experiment was the in vitro research dosages equivalent to 0.025, 0.25 and 2.5 mg/kg body weight. Based on the studies, it was concluded that the variability among individuals is small and the uncertainty factor is inexpedient to use any longer,

with the use of formula 1 has shown inconsistency of the results. This demonstrates the high uncertainty of the data, based on which it is offered to give up modifying factor 10 and the acceptable daily dose of 3 mcg/kg body weight [37,38].

II.ii. Exposure-effect assessment

Development of imbalance of intestinal microflora concentration of tetracycline in vitro data presented in Fig. 1.

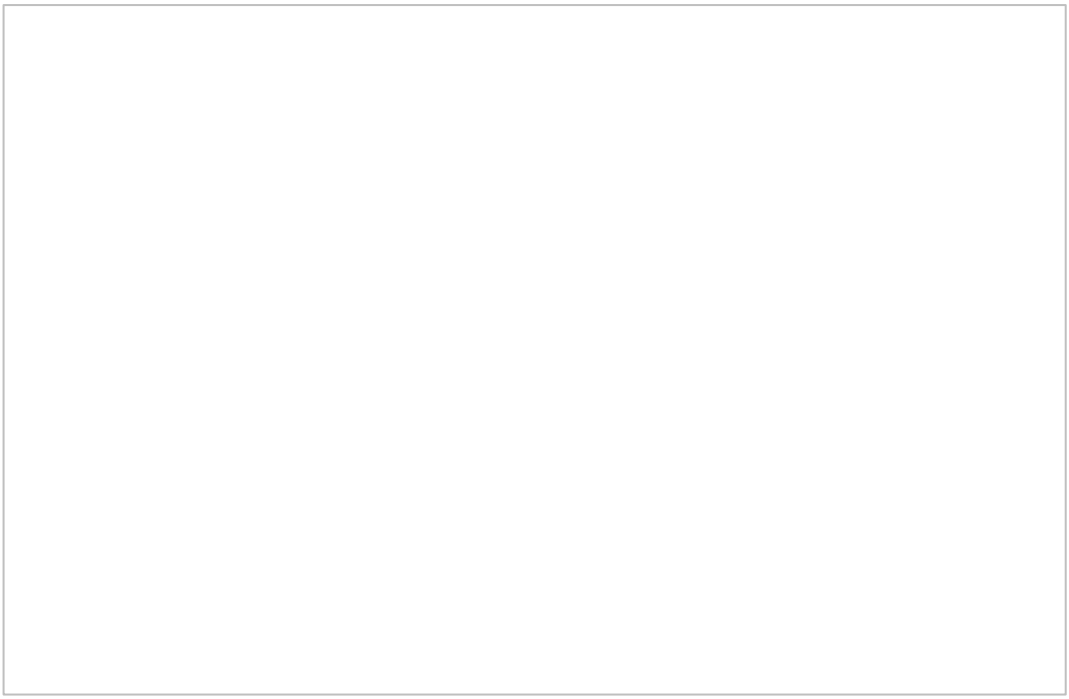
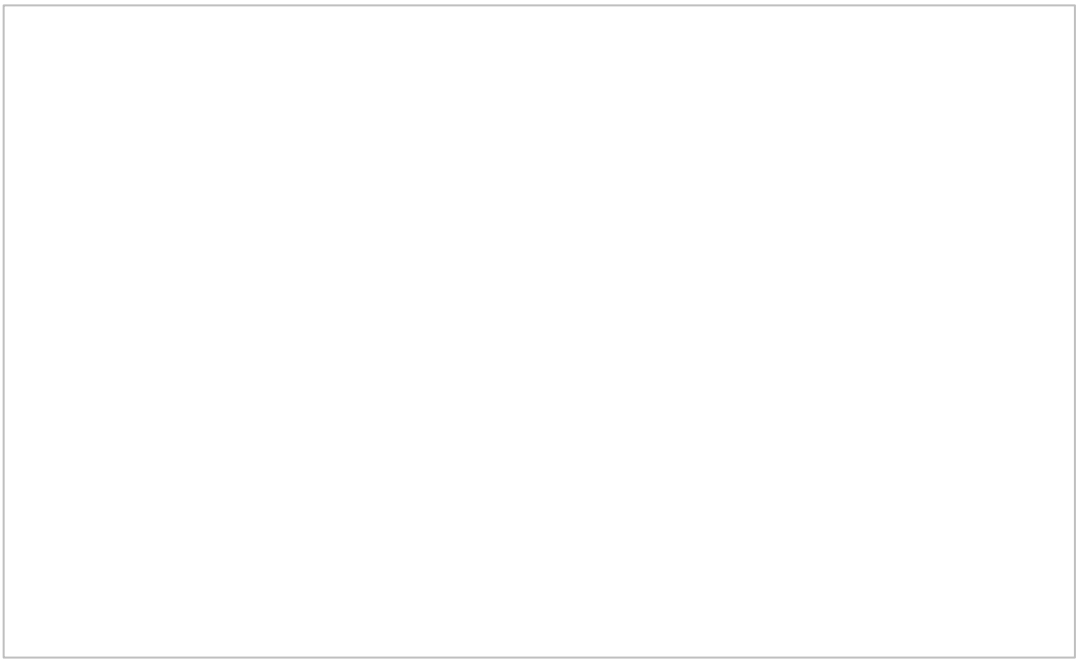


Figure 1 - The dependence of the relative abund ance of the intestinal microflora (%) on the concentration of tetracycline

Changes in the ratio of facultative and obligate intestinal flora is presented in Figure 2.

Figure 2 Comparison of obligate an d facultative intestinal microflora



Research studies on the diseases related with an imbalance of intestinal microflora in children and adults have shown that changes in the composition of the intestinal microflora for adults are: first degree dysbiosis from 18.6 to 34.8%, second degree dysbiosis - from 24.2 to 45, 7%, third degree dysbiosis - from 27.0 to 56.3%. For children: first degree dysbiosis - from 2.0 to 74.0%, second degree dysbiosis - from 26.0 to 84.0% and third degree dysbiosis - from 0.0 to 39.1% [39, 40, 45, 46].

III. EXPOSURE ASSESSMENT

Exposure assessment was based on the maximum residue levels (MRL) of tetracycline for different groups of food products of animal origin, taking into account the average daily consumption of the products.

Thus, according to the sanitary rules and regulations of the Republic of Kazakhstan in 2001, and data of "the Agency on Statistics of the Republic of Kazakhstan" the following daily maintenance of products of animal origin in the food basket for the residents of the Republic of Kazakhstan of different age groups was adopted (Table A1).

Table A1 - Daily content of products of animal origin in the food basket for the various age groups of the Republic of Kazakhstan. (g/d).

Products	Up to 3 years	3 to 7 years

Table A3 - The maximum daily dose of tetrac ycline in the daily diet for different age groups of the population of the Republic of Kazakhstan in mcg / kg by the values of the CU, WHO (1990), FAO / WHO (1998) / adopted by the Codex Alimentarius Commission and the United States

	Up to 3 years	3 to 7 years	School-age	Teenage	Adult
CU	8,29	7,89	8,12	9,27	10,84
WHO (1990)	86,87	79,45	80,85	103,70	106,60
FAO/WHO (1998)	92,17	84,75	86,15	115,70	115,60
US	184,87	393,55	388,85	421,10	542,40

With regard to the average mass of the intestinal contents spun by adults - 220g [46,47] and the average mass of intestinal contents spun by childre n (50g) [47] concentrations of tetracycline in the gastrointestinal tract are calculated (Table A4).

Table A4 - The concentrations of tetracycline in the gastro-intestinal tract for different age groups of the population of the Republic of Kazakhstan established in the CU, WHO (1990), FAO / WHO (1998) and adopted in the United States

	Up to 3 years	From 3 to 7 years	School-age	Teen-age	Adults
CU	0,17	0,16	0,16	0,04	0,05
WHO (1990)	1,74	1,59	1,62	0,47	0,48
FAO/WHO (1998)	1,84	1,70	1,72	0,53	0,53
US	7,70	7,87	7,78	1,91	2,47

Thus, the concentration of tetracycline in the gastrointestinal tract of adults ranged from 0.05 mg/g to 2.47g/g. The concentration of tetracycline in the gastro-intestinal tract of children population ranged from 0.16 mg/g to 7.87g/g.

The obtained data can be used to calculate the char acteristics of risk of forming resistant strains of micro-organisms in the gastrointestinal tract of humans and imbalance of intestinal flora to increase of pathogenic and conditionally pathogenic strains.

IV. RISK CHARACTERISTICS

The use of antibiotics contributes to the growth of resistant microorganisms, both pathogenic and opportunistic, while still maintaining hor

microflora found that values are safe for the health which are recommended by CU for all age groups in which the content of opportunistic path ogens does not exceed 5% of the total number of bacteria. The values recommended by WHO (199 0) and FAO / WHO (1998)/Codex Alimentarius are safe only for two age groups (adolescents and adults). The values on the residual tetracycline in animal products recommended by WHO (1990) and FAO/WHO (1998) for ages up to 3 years, 3 to 7 years of age and school-age children are hazardous to health.

Implementation of values recommended by WH O (1990) and FAO / WHO (1998) and adopted in the United States can be related with inhibition of obligate microflora and lead to an imbalance of intestinal microflora of varying severity (Table A7, A8 and A9).

According to data of WHO minimal inhibitory concentration of tetracycline Mic90 (concentration at

Table A6 - The change of intestinal microflora by residual tetracycline by the CU standards

Table A7 - Changing the intestinal microflora under the in fluence of residual tetracyclin e by the WHO standards (1990)

	Up to 3 years		3 to 7 years		School-age		Teenage		Adult	
Type of bacteria	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach
Bifidobacteria spp.	80,38	1,74	80,78	1,59	80,70	1,62	83,75	0,47	83,73	0,48
Bacteroides spp.	4,72	1,74	4,74	1,59	4,74	1,62	4,92	0,47	4,92	0,48
Clostridia spp.	0,01	1,74	0,01	1,59	0,01	1,62	0,01	0,47	0,01	0,48
Lactobacilli	3,78	1,74	3,80	1,59	3,80	1,62	3,94	0,47	3,94	0,48
Peptostreptokokki	1,89	1,74	1,90	1,59	1,90	1,62	1,97	0,47	1,97	0,48
E. Coli (colon bacillus)	0,97	1,74	0,98	1,59	0,97	1,62	0,99	0,47	0,99	0,48
Conditionally pathogenic microflora	8,25		7,79		7,89		4,42		4,45	

Table A8 - The change of intestinal microf lora by residual tetracycline by the FAO/ WHO (1998) and Codex Alimentarius standards

	Up to 3 years		3 to 7 years		School-age		Teenage		Adult	
Type of bacteria	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach
Bifidobacteria spp.	80,11	1,84	80,48	1,70	80,43	1,72	83,59	0,53	83,59	0,53
Bacteroides spp.	4,70	1,84	4,72	1,70	4,72	1,72	4,91	0,53	4,91	0,53
Clostridia spp.	0,01	1,84	0,01	1,70	0,01	1,72	0,01	0,53	0,01	0,53
Lactobacilli	3,77	1,84	3,79	1,70	3,79	1,72	3,93	0,53	3,93	0,53
Peptostreptokokki	1,89	1,84	1,89	1,70	1,89	1,72	1,97	0,53	1,97	0,53
E. Coli (colon bacillus)	0,97	1,84	0,97	1,70	0,97	1,72	0,99	0,53	0,99	0,53
Conditionally pathogenic microflora	8,55		8,13		8,19		4,60		4,60	

le A9 - The change of intestinal microflora by residual tetracycline regulations adopted by the US

Type of bacteria	Up to 3 years		3 to 7 years		School-age		Teenage	Adult
	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach	Share in the normal flora of the intestine,%	Number of tetracycline in the stomach		

These data indicate that the least changes in intestinal microflora are set in the process of detecting residual amounts of tetracycline in foods at 10 mg/kg (0.01 mg/kg). The greatest changes in the species composition of the normal microflora - reducing the percentage of symbiotic microorganisms and increase the number of conditionally pathogenic flora, are fixed at acceptable levels of antibiotic residues in food products to the US.

V. UNCERTAINTY OF THE RESULT ASSESSMENT

An analysis of presented materials involves the following possible uncertainties:

- The absence or incompleteness of information on the possible effects of chronic exposure of tetracycline on the human body, especially on the most sensitive population groups (children, pregnant and lactating women, people with diseases of the gastrointestinal tract, the elderly), the uncertainties related with th e transfer of the experiments carried out in vitro to humans;
- The parameters used to evaluate exposure and risk calculation (uncertainty parameters related with the consumption of food. For example, in the Republic of Kazakhstan, due to the food preferences of some gr oups of the population, the main food products are milk and meat products);
- Gaps in scientific theory required to predict on the basis of causal relationships (limited number of studies, on the basis of results of which parameters of the models has been calculated, the use of modifying factors, or no t using them, the use of generalized, averaged data for large populations, the incomplete understanding of the laws governing the formation of balance disorders microflora and related health conditions of the population. At the same time, possible underestimation of the risk to the health of the population of Kazakhstan, due to the lack of data on the consumption of offal, particularly the liver and kidneys of farm animals).

In general, the uncertainty of results of research is characterized as high.

CONCLUSION

The Republic of Kazakhstan considers that rejection of the uncertainty factor of 10 in determining the value ADI (ADI) for tetracyclines are not well grounded. Taking into account the

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