UNOFFICIAL TRANSLATION

"OO KAZAKHSKAYA ACADEMIYA PITANIYA" LLP
RSE "SCIENTIFIC-PRACTICAL CENTER OF EPIDEMIOLOGICAL INSPECTION AND MONITORING" MINISTRY OF PUBLIC HEALTH OF THE REPUBLIC OF KAZAKHSTAN

MATERIALS FOR HUMAN HEALTH RISK ASSESSMENT OF TETRACYCLINE INTAKE WITH FOOD

Almaty, 2013

TABLE OF CONTENTS:

INTRODUCTION

Tetracyclines are antibacterial drugs with broad effect 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 tetracycline were obtained in 1948-1952. The representatives of this family are characterized 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 and prevention of bacterial infections of both human and animals. In animal husbandry, they are 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. Adding 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 European Union, the US, norms and standards of the Codex Alimentarius, which are significantly 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 values (2,0 - 8,5), rapid absorption in the gastrointestinal tract, higher blood concentration.

Tetracyclines antibacterial action is based on the suppression of protein synthesis due to defect of the complex formation between the transfer RNA and ribosome.

Tetracyclines are active against Gram-positive microorganisms: Staphylococcus spp. (including Staphylococcus aureus, including penicillinase-producing strains), Streptococcus spp. (including Streptococcus pneumoniae), Listeria spp., Bacillus anthracis, Clostridium spp., Actinomyces israelii; Gram-negative microorganisms: Haemophilus influenzae, Haemophilus ducreyi, Bordetella

pertussis, the majority of Enterobacteriaceae: Escherichia coli, Enterobacter spp., including Enterobacter aerogenes, Klebsiella spp ., Salmonella spp., Shigella spp., Yersinia pestis, Bartonella bacilliformis, Vibrio cholerae, Vibrio fetus, Rickettsi spp., Borrelia burgdorferi, Brucella spp., and also are active against pathogens lymphogranuloma venereum and groin, Treponema spp. Tetracycline-resistant microorganisms: Pseudomonas aeruginosa, Proteus spp., Serratia spp., Most strains of Bacteroides spp. and fungi, viruses, beta-hemolytic group A streptococci (including 44% of Streptococcus pyogenes strains and 74% of strains Streptococcus faecalis) [7.].

I.1. Distribution throughout the body

Tetracycline is well absorbed with a parenteral administration, many organs and tissues. Does not permeate effectively through the intact blood-brain barrier, but in the case of diseases of brain and its membranes permeating into the spinal fluid flow increases significantly. It is binding with plasma protein up to 65%. Selectively accumulates in bones, teeth, liver, spleen, and in significant amount in the tumor tissues. It is effectively permeating through the placental barrier and is excreted through breast milk.

Following injection, 60-80% of the dose is absorbed in the gastrointestinal tract. Unchanged form excreted in the urine and feces (in feces 20-50%, urine 20-69%). In case of impaired renal excretory function , tetracycline concentration increases in the blood, which can lead to appearance of accumulation. [8]

The elimination half-life of lipophilic doxycycline is 2-3 times longer than of tetracycline. Despite the good absorption in the digestive tract, tetracycline residual concentration in intestine is always sufficient to affect the intestinal microflora [7,10].

I.2. Main Effects

Peak plasma concentrations (up to 3.6 mg/L) were detected after administration to white rats a single dose of tetracycline 75 mg/kg after 2 hours, decreasing to 0.5 mg/L after 6 hours. Peak concentrations in liver and kidney were detected in 2 hours after administration [9].

Tetracycline hydrochloride (10 mg/kg) was administered to dogs intravenously and the highest level was found in liver and kidneys. In case of oral administration to the dogs in a dose of 25 mg/kg, the peak concentration was 3 mg/ml after 2 hours, decreasing to 0.27 after 24 hours [11].

Antibiotic excretion occurs predominantly in the urine. 69.2% and 19.5% of the antibiotic was detected in the urine within 72 hours after intravenous administration of tetracycline in two rats and one dog at a dose of 15 mg or 4 mg/kg [9].

Tetracyclines high doses at the level of 2,500 to 7,500 mg kg of body weight can induce weight loss and limited fat change in the liver of rats [12]. Gonadotoxic, embryotoxic, teratogenic, mutagenic and carcinogenic effects in studied doses have not been identified. [13] It is noted that an increase in the dose of the drug increased the fraction of tetracycline in the bones and pigmentation [14].

In human body tetracycline is well absorbed in the gastrointestinal tract (66% of the dose). Maximum concentrations in blood serum are detected within 1-3 hours after ingestion. Higher concentrations are achieved by intravenous administration.

Absorption of tetracycline antibiotics differ in human and animals, setting up only 4-9% in mice and rats [13]. Significant differences in indicators between human and animals are:

- quantity of antibiotics absorbed in the gastrointestinal tract;
- time which take the body accumulation to peak concentration of antibiotic;
- localisation of peak concentrations of the antibiotic in the body;
- time for exerting the antibiotic from the body.

These differences must be considered when extrapolating the results of experiments detected with laboratory animals to human.

Ingestion of the antibiotic in the human body is not determined by its effect on the influence of pathogens, but involves obligate and facultative intestinal microflora, inducing a state of dysbiosis. Antibiotics also interact with the human body depending on the dose that causes the development of adverse changes in metabolic, functional and morphological level [15].

The major health concern is the development of resistance to antibiotics and the subsequent transport of the tetracycline resistance strains of microorganisms to man. The risk of infection of the food stock by antibiotics residues is directly linked to the development of microbial resistance. Not only livestock and poultry are infected by antibiotic, but also plant production. Antibiotics exerted from the animals come as organic fertilizer to the soil and then continue to accumulate in plant foods. Polyresistance to antibiotic now shows up at the representatives of pathogenic, as well as opportunistic microorganisms. Very often the cause of hospital infections and food poisoning are salmonella. Among cultures of Salmonella isolated from humans, resistance to antibiotics occurred more frequently than in cultures isolated from farm livestock. Tetracycline resistance was detected in 49.1% [16]. It was shown that among clinical strains of microorganisms, enterobacteria had the highest percentage of antibiotic resistance. Among the Gram-negative microorganisms are the most common strains show resistant to 9-11 drugs, which is confirmed by data obtained by the Kazakh Academy of Nutrition from 1986 to 1999. Antibiotic-resistant bacteria are founded almost in feces of all people that can selectively survive when contact with medication [18].

Normal microflora, as a human symbiont, is critical to the functioning of the body and ensures its homeostasis.

The proportion of the intestinal microflora may change under the influence of tetracycline, until the complete disappearance of some or almost all of the Indigenous (autochthonous) strains and starts the rapid proliferation of opportunistic bacteria. Changes of such nature are called dysbiosis [20, 21, 22, 23, 24, 25].

In clinical practice, to the 10th revision of the International Classification of Diseases [26] has identified a number of diseases related to imbalance of the intestinal microflora: irritable bowel syndrome (IBS) with diarrhea (K58.0), without diarrhea (K58.9), constipation (K59.0), inflammatory bowel disease (IBD) (K50-K51).

Microflora imbalance can often be a trigger for the development of pathology in many different systems and organs, as well as reason of secondary immunodeficiency states and complicate the course of various diseases of infectious and noninfectious etiologies. [27,28,29,30,31,32,33].

It is believed that, besides the above-mentioned diseases functional bowel disorders (K59.9), duodenitis (K29.8, K29.9), food allergy (T78.0, T78.1, T78.4), including atopic dermatitis (L20.8), iron deficiency anemia (D50), a general immunodeficiency (D83.9) [34,35,36] could be developed in children with an imbalance of intestinal microflora.

Exposure of human body to residues of antibiotics, in particular, of the tetracycline group, can be attributed to chemical factors of low intensity. Such body burden can induce long-term effects, and reduce the body's resistance to adverse environmental and socio-related factors.

In general, there are many scientific evidences of underestimation in the depth of the effect of low doses of biologically active xenobiotics of anthropogenic origin. First of all it applies to tetracyclines, which are affecting human environment, reduce adaptive capacity of people, reducing the performance of their sanitary and epidemiological welfare. [37]

II. HAZARD CHARACTERISTICS

II.1. Permissible exposure limit

In 2006, the Expert Committee on Food Additives, FAO/WHO tolerable daily dose of tetracyclines has been revised from 0-3 mcg per kg of body weight per day to 0-30 mcg per kg of body weight per day [26]. In 1990, when establishing the acceptable daily dose (0-3 mcg per kilogram body weight per day) 10 uncertainty factor was accepted due to the variability of the intestinal flora of humans. It was assumed that the real tolerable daily dose may be somewhat too high.

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, and therefore, a daily dose of 0-30 mkg per kg of body weight per day shall be established [37].

However, such decision is unreasonable for the reasons stated by Russian FSFI "Federal Research Center of Medical and Preventive Risk Management Techniques to Public Health".

1. Tolerable daily dose is set for conditions of consumption of foods daily over a lifetime and cannot be based on data obtained in the short-term in vitro experiment without proper uncertainty analysis that takes into account the transfer of results of short-term studies obtained in vitro for human to the effects over a lifetime [28, 29].

2. In accordance with formula (1) recommended by FAO / WHO [29] tolerable daily doses of tetracycline and oxytetracycline for various types of microorganisms twere calculated.

$$AD = \frac{MIC_{50} \text{ x Mass of intestinal contents (220g)}}{\text{dose inhibiting the growth pf microorganisms upon preoral introduction}} \times \text{margin factor} \times \text{weight of the individual (60g)}$$

The formula was developed on the basis of modal values MIC50 (MIC50 - minimal concentration of the antibiotic which inhibits growth of 50% of the cultures certain microorganism), factor margin to account for various variability, mass of the intestinal contents, weight and individual oral dose of antibiotic to inhibit growth of microorganisms.

MIC50 values of tetracycline and oxytetracycline for 10 different microorganisms were taken in accordance with the WHO Food Additives Series 36, the stock value of the factor - 1, a dose that inhibits the growth of microorganisms in the oral administration of tetracycline - 0.6 mg, the mass emitted intestinal contents - 220g, average weight individual - 60 kg.

In accordance with formula (1), a tolerable daily dose of tetracycline derived from the minimum inhibitory concentrations (MIC50) for some microorganisms varied from 0.37 mg/kg body weight against Clostridium spp. to 195.6 mg/kg body weight for the Escherichia coli and Proteus spp. (Table 1). This high variability of the results may indicate the need to include in the calculation of the acceptable daily dose of additional uncertainty, especially for the most sensitive populations such as children. The characteristics of these groups shall be taken into account in the assessment of health risks, including the immaturity of the local intestinal immunity in infants, the presence of chronic gastrointestinal diseases, altered immune status, violations of microbocenosis GI, dietary habits of the population.

Table 1

Microorganism	Dose causing inhibition of the growth of microorganisms mcg / kg
Escherichia coli	195,56
Bifidobacterium spp.	97,78
Bacteroides fragilis	24,44
Eubacterium spp.	12,22
Clostridium spp.	0,38
Streptococcus spp.	97,78
Fusobacterium spp.	0,76
Lactobacillus spp.	12,22
Proteus spp.	195,56
Peptostreptococcus spp.	12,22

3. The reason for not using a factor of uncertainty in setting ADI of tetracyclines was the results of an experiment in vitro [26]. It was shown that concentrations equivalent doses of 0.025, 0.25 and 2.5 mg/kg body weight were studied, and findings suggested that the dose of 0.025 and 0.25 mg/kg body weight has no effect. It is not possible to establish how dose equivalence of levels of exposure to microflora chemostat was assessed. Evaluation of the proposed correlation

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.

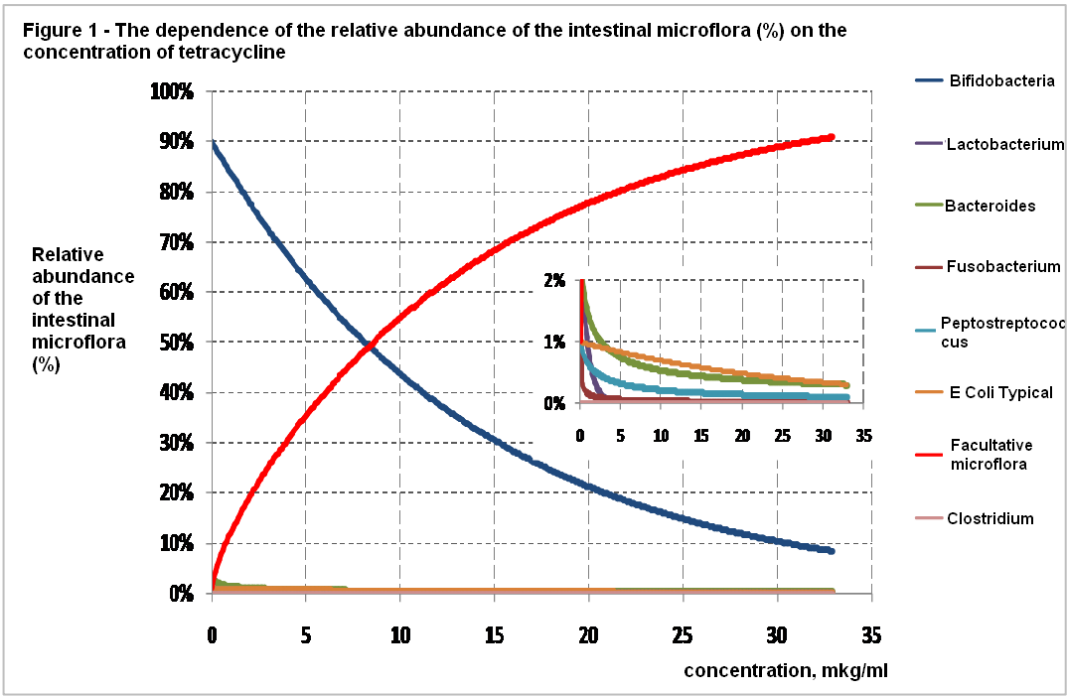
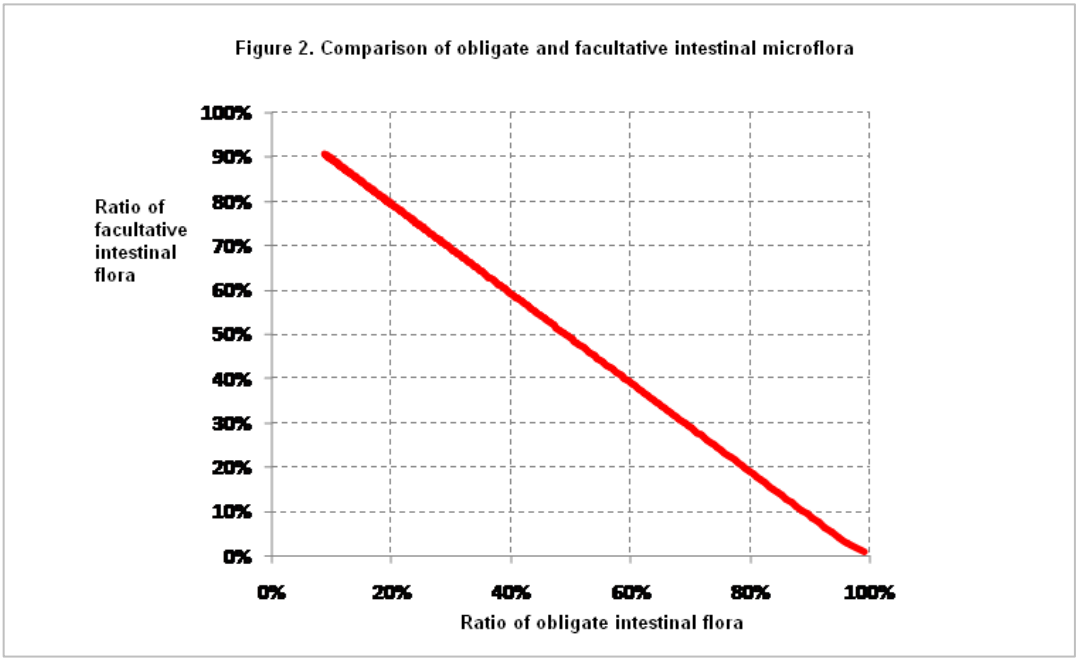


Figure 1 - The dependence of the relative abundance of the intestinal microflora (%) on the concentration of tetracycline

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

Figure 2 Comparison of obligate and 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	School-age	Teenage	Adult
Meat and meat products	65	100	95	100	150
Milk, milk products	635	570	570	592	579
Poultry and poultry products	17	17	25	30	55
Egg	15	27	27	55	38
Fish and Seafood	15	50	60	100	185
Oils and fats of animal origin	17	25	35	50	70
Total	764	789	812	927	1084

The values of maximum residue levels of tetracycline for adults to various animal tissues and types of exposure recommended by the WHO (1990), FAO/WHO (1998) and adopted in the United States (Table A2).

Table A2 - MRL values for different types of animal tissues and exposure

Food product	CU	WHO 1990	FAO/WHO 1998, Codex Alimentarius	USA
Milk, mcg/l	10	100	100	300
Muscle tissue, mcg/l	10	100	200	2000
Fat tissue, mcg/l	10	10	-	10
Egg, mcg/l	10	200	400	200
Liver, mcg/l	10	300	600	300
Kidney, mcg/l	10	600	1200	600

On the basis of the data presented maximum daily dose of tetracycline with food was calculated for different age groups of the population of Kazakhstan (Table A3).

Table A3 - The maximum daily dose of tetracycline 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 children (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 characteristics 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 high bacteriostatic and bactericidal activity of drugs in relation to obligate anaerobic flora (Bifidobacterium, Bacteroides, Clostridium, fuzobakterium, peptostreptokoks, lactobacillus). The level of pathogenic (commensal) flora (Escherichia coli, Salmonella spp., Campylobacter spp. And Enterococci) - is a predictor of induction of bacterial resistance against antibiotics and an indirect measure of the expected resistance of pathogens. Monitoring observations of resistant commensals (Escherichia coli and Enterococci) is an indicator of the prevalence of antibiotic resistance in the population [19].

Emerging changes in the spectrum of microbial flora of the intestine against the effects of tetracycline as a violation of the relationship between aerobic and anaerobic flora with the suppression of the growth of obligate microorganisms (Lactobacillus fuzobakterium, peptostreptokoks, Clostridium) leads to an increase in the proportion of Escherichia coli, Salmonella spp., Campylobacter spp. and Enterococci, Staphylococci, indicating the unbalance of the intestinal microflora [19, 23, 24, 25, 26].

Assessment of health risks when exposed to tetracycline, coming from the food products, made separately for each age group. The calculations were made on maximum residue limits of tetracycline for four values recommended by the CU, WHO (1990), FAO / WHO (1998) and adopted in the United States (Table A4). In the simulation result in imbalance of intestinal

microflora found that values are safe for the health which are recommended by CU for all age groups in which the content of opportunistic pathogens does not exceed 5% of the total number of bacteria. The values recommended by WHO (1990) 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 WHO (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 which 90% of bacteria is inhibited) has been installed. Table A5 contains the normal microflora composition and the data of minimum inhibitory concentrations of tetracycline Mic90 [13].

Table A5 - MIC90 values of tetracycline against the human intestinal microflora

Type of bacteria	Share in the normal flora of the intestine,%	Mic_{90}, mcg/ml
Bifidobacteria spp.	85	32
Bacteroides spp.	4,99	32
Clostridia spp.	0,01	32
Lactobacilli	4	32
Peptostreptokokki	2	32
E. Coli (colon bacillus)	1	64

The results of calculations of parameters for each type of bacteria of the intestinal microflora, for each age group, according to the norms recommended by the CU, WHO (1990), FAO/WHO (1998) and adopted in the United States are given in Tables A6, A7, A8 and A9.

Table A6 - The change of intestinal microflora by residual tetracycline by the CU 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.	84,55	0,17	84,58	0,16	84,58	0,16	84,89	0,04	84,87	0,05
Bacteroides spp.	4,96	0,17	4,97	0,16	4,97	0,16	4,98	0,04	4,98	0,05
Clostridia spp.	0,01	0,17	0,01	0,16	0,01	0,16	0,01	0,04	0,01	0,05
Lactobacilli	3,66	0,17	3,68	0,16	3,68	0,16	3,92	0,04	3,90	0,05
Peptostreptokokki	1,99	0,17	1,99	0,16	1,99	0,16	2,00	0,04	2,00	0,05
E. Coli (colon bacillus)	1,00	0,17	1,00	0,16	1,00	0,16	1,00	0,04	1,00	0,05
Conditionally pathogenic microflora	3,83		3,78		3,78		3,20		3,24	

Table A7 - Changing the intestinal microflora under the influence of residual tetracycline 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 microflora 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	

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

	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.	64,55	7,7	64,10	7,87	64,33	7,78	79,93	1,91	78,44	2,47
Bacteroides spp.	3,79	7,7	3,76	7,87	3,78	7,78	4,69	1,91	4,60	2,47
Clostridia spp.	0,01	7,7	0,01	7,87	0,01	7,78	0,01	1,91	0,01	2,47
Lactobacilli	3,04	7,7	3,02	7,87	3,03	7,78	3,76	1,91	3,69	2,47
Peptostreptokokki	1,52	7,7	1,51	7,87	1,51	7,78	1,88	1,91	1,85	2,47
E. Coli (colon bacillus)	0,88	7,7	0,88	7,87	0,88	7,78	0,97	1,91	0,96	2,47
Conditionally pathogenic microflora	26,22		26,73		26,46		8,76		10,45	

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 the 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 groups 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 not 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 characteristics of the consumption of meat and dairy products in the country, the establishment of acceptable residual amounts of tetracycline at 100 mcg/l of milk, 100 mcg/kg in muscle tissue, 10 mcg/kg in fat tissue, 200 mcg/kg for eggs, 300 mcg/kg liver, 600 mcg/kg in the kidneys could lead to additional risk of digestive diseases, anemia, dermatitis, allergy and immunodeficiency in the most sensitive population groups (children, pregnant and lactating women, people with diseases of the gastrointestinal tract, the elderly).

The Republic of Kazakhstan maintains its position and believes that the establishment of allowable residues of tetracyclines in food at a level of 10 mcg/kg (0.01 mg/kg) was justified and would not increase the risk to public health.

Literature used

1. Antibiotics chemical, 3rd ed., volume 1, Moscow, 1961, p. 180-268;
2. Nazashin S.M, Fomina I.P., Rational antibiotic therapy, 4th ed., Moscow, 1982, p.183-208.
3. Chernukh A.M., Kivman G.Y., Tetracycline antibiotics, M., 1962.
   Fighting against antibiotics resistance from position of safety of food products in Europe, WHO Regional Office for Europe, p. 1, 2011
4. Chepurnoy I.P. Nutrition and health. 3rd ed. Moscow, 2008, p. 208
5. 9th Moscow International Veterinary Congress, speaker A.N.Panina, FGBU "All-Russian State Centre for Quality and Standardization of Veterinary Drugs and Feed," 18April 2011.
6. Practical guide on anti-infective chemotherapy, Editors: L.S. Stratchounski, Y.B. Belousov, S. N. Kozlov, Smolensk: MARMAX, 2007. 464 p.
7. Mashkovsky M.D. " Medicinal products" ed. 13, v.2, Kharkov, 1998, p. 258
8. Chlortetracyclin and Tetracyclin. First draft prepared by M.F.A. Wouters, J.E.M.
9. van Koten-Vermeulen, F.X.R. van Leeuwen of Toxicology Advisory Centre National Institute of Public Health and Environmental Protection. WHO Food Additives Series: 36. - Bilthoven, Netherlands, 1998
10. Navashin S.M., Fomina I.P., Reference book on Antibiotics, 3rd ed., Moscow, 1974

11. Kelly, R.G. (1964) Antibacterial tetracyclines.In tissue distribution of tetracycline and chlortetracycline. American Cyanamid Company Report P.R. 9:485-492 (Pearl River). Submitted to WHO by American Cyanamid Company, Princeton, USA.
12. NTP Toxicology and Carcinogenesis Studies of Tetracycline Hydrochloride (CAS No. 64-75-5) in F344/N Rats and B6C3F1 Mice (Feed Studies), 1989.
13. Chlortetracyclin and Tetracyclin. First draft prepared by M.F.A. Wouters, J.E.M. van Koten-Vermeulen, F.X.R. van Leeuwen of Toxicology Advisory Centre National Institute of Public Health and Environmental Protection. WHO Food Additives Series: 36. - Bilthoven, Netherlands, 1998.; European Community Comments for the CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS Washington, D.C., 28 - 31 March 2000 CL 1999/13 GEN.
14. Evaluation of certain veterinary drug residues in food, WHO, 1990.
15. Deichmann, W.B., Bernal, E., Anderson, W.A.D., Keplinger, M., Landeen, K., Mcdonald, W., Mahon, R. & Stebbins, R. (1964) The chronic oral toxicity of oxytetracycline HCl and tetracycline HCl in the rat, dog and pig. Ind.Med.Surg., 787-806.
16. Mikhalevsky N. P "Influence of penicillin and tetracycline on dynamics of content of amino acids, nucleic acids and general protein in the endocrine glands" 1983, Tselinograd, p. 158.
17. Rozhnova Sh., Sechkarova A., Yanushkova Yu., Prikazska K., Golotova E., Soloveva N.K. Some aspects of drug resistance of Salmonella / / ZhMEI.-1991. - № 8. - P.27-29
18. Hadzhibaeva I.F. "Developing ways to ensure stability of microbial indicators of food, improving methods of monitoring their security," Thesis. ... Cand. Sc. {Biology}, RK, 2006 p.12.
19. Van den Bogaard A.E., Stobberingh E.E. Epidemiology of resistance to antibiotics. Links between animals and humans. Int J Antimicrob Agents. 2000 May;14 (4):327-35.
20. Shcherbakov P.L., Kudryavtseva L.V., Zaitseva S.V., Petrova N.N., et al. Microbiocenosis of intestinal: its disorders and correction with use of Bactisubtil/ / Pediatrics. - 1998. - 5. P. 99-103.
21. Vorobyeva A.A., Abramov N.A., Bondarenko V.M., Shenderov B.A. Dysbacterioses - current issue of Medicine / / Journal of RAMS. - 1997. - 3. P.4-10.
22. Bondarenko V.M., Petrovskaya V.G. Early stages of infection process and dual role of normal microflora / / Journal of RAMS. - 1997. - 3. P. 7.10.
23. Lisbeth Elvira de Vries, Henrik Christensen1, Robert L. Skov, Frank M. Aarestrup and Yvonne Agersø. Diversity of the tetracycline resistance gene tet(M) and identification of Tn916- and Tn5801-like (Tn6014) transposons in Staphylococcus aureus from humans and animals. J of Antimicrobial Chemotherapy, 2009.
24. Yvonne Agersø, Dominguez, E., M. Zarazaga, Y. Saenz, L. Brinas, and C. Torres. 2002. *Mechanisms of antibiotic resistance in Escherichia coli isolates obtained from healthy children in Spain.* Microb. Drug Resist.8**:**321-*327.*
25. Nowrouzian F, Hesselmar B, Saalman R, Strannegard IL, Aberg N, Wold AE, Adlerberth I. Escherichia coli in infants' intestinal microflora: colonization rate, strain turnover, and virulence gene carriage. Pediatr Res. 2003 July;54(1):8-14.
26. Van den Bogaard AE, Stobberingh EE. Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs. 1999 Oct;58(4):589-607.
27. Savelyeva L.A., Butenina E.M., Lebedeva E.M., Akulova F.D. Actual issues of clinical microbiology. - M., 1985. - P. 21-25.
28. Leonard E.M., Van Saene H.K.F., Shears P. et al. Pathogenesis of colonization and infection in a neonatal surgical unit // Crit. Care Med.-1990.-18, 3, 264-269.
29. Pryamukhina N.S., Semina N.A. Differentiation of intestinal Escherichiosis / / ZhMEI. - 1991, 2. P.81-87.
30. Gizatulina S.S., Birger M.O., Nikovskaya M.I. et al. Intestinal microflora of infants with rotavirus infection / / ZhMEI. - 1992. - 3. P. 29-33.
31. Krasovskaya T.V., Beloborodova V.P. Surgical infection of newborns. - M., 1993. - P. 18-27.
32. Slabospitskaya A.T., Vinogradova V.P., Krymovskaya S.S., et al. New drug biosporin and its effect on intestinal microflora at dysbacteriosis of newborns / / Journal of Microbiology. - 1995. - 1. p. 71-76.
33. Jukes TH: Public health significance of feeding low levels of antibiotics to animals .AdvApplMicrobiol 16:1-30, 1973.
34. Kamalova A.A. Microecology condition of gastrointestinal tract in children with chronic gastroduodenal pathology: Thesis ... M.D.Kazan. 2011. - 267 p.
35. Gorodkova E.N. Some metabolic parameters at irritable bowel syndrome associated with connective tissue dysplasia in children and justification of the combined therapy method: Thesis. ... Candidate of Medical Science. Saratov, 2007. - 137 p.
36. Komarova E.V. Chronic constipation in children: medical and social aspects: Thesis M.D. Moscow. 2007. – 215 p.
37. Updating the Principles and Methods of Risk Assessment: MRLs for Pesticides and Veterinary Drags, Rome, 2006.
38. Onishchenko G.G., Sheveleva S.A., Khotimchenko S.A "Hygienic substantiation of permissible levels of tetracyclines in food" "Hygiene and Sanitation" Journal, 2012, № 6, p.4-14
39. Onishchenko G.G., Sheveleva S.A., Khotimchenko S.A. "New aspects of safety assessment and food contamination by tetracycline antibiotics in the light of harmonization of sanitary hygienic norms of Russian legislation and the Customs Union with international standards," "Nutrition" Journal, 2012, Vol 81, № 5, p.4-12
40. Vol. 8 Notice to applicants and notes for guidance (Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin), Belgium, 2003.

41. Prakash S, Rodes L, Coussa-Charley M, Tomaro-Duchesneau C. Gut microbiota: next frontier in understanding human health and development of biotherapeutics. Biologics. 2011; 5: 71–86.
42. DoréJ, CorthierG. [The human intestinal microbiota]. Gastroenterol ClinBiol. 2010 Sep;34 Suppl1:S7-15.
43. Review Colonization and impact of disease and other factors on intestinal microbiota. Thompson-Chagoyán OC, Maldonado J, Gil A. DigDisSci. 2007 Sep; 52(9):2069-77. Epub 2007.Apr. 10.
44. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 38, 1996
45. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 41, Tetracycline: Oxytetracecline, Chlortetracecline fnd Tetracycline (addendum) //IPCS – International Programme on Chemical Safety // The 5-th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). - World Health Organization, Geneva, 1998.
46. Studies to evaluate the safety of residues of veterinary drugs in human food: general approach to establish a microbiological ADI, London, 2007.
47. Clinical laboratory tests from A to Z and their diagnostic profiles / Kamyshnikova V.S.- Moscow: MED Press Inform, 2007. – p. 320.

_____