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Research article

ON SUFFICIENT SUBSTANTIATION FOR MAXIMUM PERMISSIBLE LEVEL OF ZILPATEROL IN MEAT PRODUCTS

S.E. Zelenkin[1], P.Z. Shur[1], D.A. Kiryanov[1], V.M. Chigvintsev[1], O.Yu. Ustinova[1], V.A. Fokin[1], D.V. Suvorov[1], E.V. Fedorenko[2]

[1]Federal Scientific Center for Medical and Preventive Health Risk Management Technologies, 82 Monastyrskaya Str., Perm, 614045, Russian Federation
[2]Scientific-Practical Hygiene Center, 8 Akademicheskaya Str., Minsk, 220012, Republic of Belarus

The Joint FAO/WHO Expert Committee recommends the maximum permissible level of zilpaterol in meat to be fixed at 0.5 μg/kg. This level is substantiated by results of analysis described in several research works. Nevertheless, substantiation provided for this recommended standard requires a detailed discussion.

In this study, we aimed to analyze substantiation of FAO/WHO suggestions on the maximum permissible level (MPL) of zilpaterol in meat as per health risks for consumers.

Our analysis of research results revealed that the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) were established allowing for negative effects on various organs and systems in the body. The lowest observed adverse effect level (LOAEL) under acute exposure was taken as a baseline for establishing MPL. This level produces negative effects on the nervous system (developing tremor). However, modifying factors used in MPL development have not been supported with solid argument. We also established that the LOAEL identified for the nervous system under acute exposure was much lower than NOAELs for other organs and systems under chronic exposure. Therefore, the aforementioned research results seem rather controversial.

It is necessary to consider another additional factor, which is wide prevalence of cardiovascular diseases among adult population and risk factors that cause their development. Therefore, potential adverse effects on the cardiovascular system are no less important and we should note that they have been reliably detected both in acute and chronic experiments.

In this study, we modeled a health risk caused by adverse effects of consuming meat products with residual zilpaterol levels; the risk was modeled in dynamics. The modeling experiment established that an impermissible health risk of adverse health outcomes in the cardiovascular system occurred even under exposure to zilpaterol in levels close to the lowest limit of sensitivity. Consequently, it seems rather premature to accept the maximum permissible level for zilpaterol in meat that is being suggested at present. It is recommended to cut its level down to the lowest limit of detection.

Keywords: *zilpaterol, food products, meat products, risk assessment, maximum permissible level, LOAEL, NOAEL, mathematic modeling.*

Sergey E. Zelenkin – Junior Researcher at the Health Risk Analysis Department (e-mail: zelenkin@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0002-0259-5509).

Pavel Z. Shur – Doctor of Medical Sciences, Chief Researcher-Academic Secretary (e-mail: shur@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0001-5171-3105).

Dmitrii A. Kiryanov – Candidate of Technical Sciences, Head of the Department for Mathematical Modeling of Systems and Processes; Associate Professor at the Department of Human Ecology and Life Safety (e-mail: kda@fcrisk.ru; tel.: +7 (342) 237-18-04; ORCID: https://orcid.org/0000-0002-5406-4961).

Vladimir M. Chigvintsev – Candidate of Physical and Mathematical Sciences, Researcher at the Situation Modeling and Expert and Analytical Management Techniques Laboratory (e-mail: cvm@fcrisk.ru; tel.: +7 (342) 237-18-04; ORCID: https://orcid.org/0000-0002-0345-3895).

Ol'ga Yu. Ustinova – Doctor of Medical Sciences, Deputy Director responsible for Clinical Work (e-mail: ustinova@fcrisk.ru; tel.: +7 (342) 236-32-64; ORCID: https://orcid.org/0000-0002-9916-5491).

Vladimir A. Fokin – Researcher at the Health Risk Analysis Department (e-mail: fokin@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0002-0539-7006).

Dmitrii V. Suvorov – Junior Researcher at the Health Risk Analysis Department (e-mail: suvorov@fcrisk.ru; tel.: +7 (342) 238-33-37; ORCID: https://orcid.org/0000-0002-3594-2650).

Ekaterina V. Fedorenko – Candidate of Medical Sciences, Associate Professor, Deputy Director responsible for support of practical sanitary-epidemiologic surveillance and work with the Eurasian Economic Commission (e-mail: afedorenko71@mail.ru; tel.: +375 (17) 284-13-70; ORCID: http://orcid.org/0000-0003-1240-1234).

At present, growth promoters are used in animal husbandry worldwide to enhance productivity. Along with hormonal stimulators (stilbenes or steroid hormones), non-hormonal growth promoters are also of practical interest. Among them, special attention is paid to beta-adrenergic agonists (ractopamine, zilpaterol, clenbuterol). Zilpaterol is the most interesting beta-agonist as regards its safety for human health.

In some countries, zilpaterol (in the form of zilpaterol hydrochloride) is applied as a food additive [1]. The basic purpose of its usage is to enhance muscle growth and increase carcass leanness; another aspect is making feed usage more effective (muscle growth enhancement due to using a food additive) in livestock breeding [2].

At present, zilpaterol hydrochloride as a food additive is considered safe for consumers in some countries (Brazil, Canada, Guatemala, Honduras, Nicaragua, Peru, the USA and some others) [3] whereas China and Taiwan prohibit to use it in agriculture and animal husbandry [4]. In the European countries, use of β-agonists, zilpaterol included, has been allowed since 1996 only for therapeutic use under direct veterinary supervision. Nevertheless, its residual quantities in meat and meat products are not regulated[1].

Zilpaterol can produce certain effects on β-adrenergic receptors of skeletal muscles and smooth muscles of the bronchi, uterus, heart, vessels and other organs [5–8]. Adverse effects on the human body caused by zilpaterol intake are associated with its pharmacological activity and can involve influence on the cardiovascular system (CVS) (with developing heart failure and future decrease in life expectancy at birth [9–12]), respiratory system, nervous system and also produce some systemic effects (a rise in the body temperature in particular) [13]. That is, when zilpaterol is administered with food even in residual quantities, it can create unacceptable (impermissible) public health risks.

Given that, in 2016 the Joint FAO/WHO Expert Committee on Food Additives (JECFA), relying on the analysis of some research works, recommended the maximum permissible level (MPL) of zilpaterol in meat to be fixed at 0.5 μg/kg [14]. Nevertheless, substantiation provided for this recommended standard requires a detailed discussion.

In this study, we aimed to analyze substantiation of FAO/WHO suggestions on the maximum permissible level (MPL) of zilpaterol in meat as per health risks for consumers.

Our analysis revealed that the JECFA relied on the results obtained by studies with their focus on acute effects; those studies were conducted with participating volunteers, asthmatic patients included (as the most sensitive population group). The results allowed establishing no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) allowing for adverse effects on various organs and systems in the body (Table 1). The LOAEL for cardiovascular effects was established to be equal to 0.25 mg/person; respiratory effects, 0.1 mg/person; effects on the nervous system, 0.05 mg/person. In its turn, the NOAEL for cardiovascular effects amounted to 0.1 mg/person and for respiratory effects, 0.05 mg/person. The experts failed to establish a NOAEL that could cause adverse effects on the nervous system within the analyzed exposure limits [14].

Chronic experiments on animals also made it possible to establish NOAELs and LOAELs for cardiovascular effects, blood and systemic effects (Table 2). Thus, the LOAEL

[1] Council Directive 96/22/EC of 29 April 1996 concerning on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/299/EEC. *Official Journal of the European Communities*, 1996, no. L 125/3, pp. 3–9.

Table 1

Research results obtained by the JECFA regarding influence exerted by zilpaterol on the human body under oral administration

Critical effect	NOAEL, mg/person*.	LOAEL, mg/person
Cardiovascular system:	0.10	0.25
Systolic blood pressure (elevated)	0.25	0.50
Diastolic blood pressure (elevated)	0.25	0.50
Cardiac output (elevated)	0.25	0.50
Elevated heart rate	0.10	0.25
The respiratory system: Bronchodilation	0.05	0.10
The nervous system: **Tremor**	–	**0.05**

Note: * means body weight is considered as equal to 70 kg.

Table 2

Research results regarding effects produced by zilpaterol on the body under chronic exposure

Species	Critical effect	NOAEL, mg/kg of body weight a day	LOAEL, mg/kg of body weight a day
Mice	Changed enzyme ratios in blood	0.02	0.05
Rats	Decreased heart rate	–	0.05
	Growing body weight	–	0.06
	Hypersalivation, growing body weight	0.2	2.0
Rabbits	Growing body weight	–	20.0
Dogs	Peripheral vasodilatation, increased heart rate, lower blood pressure	–	0.5
Javanese monkey	Increased heart rate	0.01	0.05

that causes adverse effects on the CVS and blood was established to be equal to 0.05 mg/kg of body weight; systemic effects, between 0.06 and 20.0 mg/kg of body weight. The NOAEL established for cardiovascular effects equaled 0.01 mg/kg of body weight; blood, 0.02 mg/kg of body weight; systemic effects, 0.2 mg/kg of body weight. The JEFCA materials do not provide any information on exploring chronic effects produced by zilpaterol on the nervous system.

Therefore, this information provided by the JEFCA indicates that no NOAEL was established for effects on the nervous system under acute exposure. At the same time, there are no data on negative effects produced on the nervous system by zilpaterol within the analyzed levels under chronic exposure.

The LOAEL for the nervous system under acute exposure was also established to be considerably lower than NOAELs established for other organs and systems under chronic expo-

sure. Therefore, the aforementioned research results seem rather controversial. Nevertheless, the LOAEL that could cause adverse effects on the nervous system (developing tremor) under acute exposure was taken as a starting point to derive the MPL for zilpaterol. This dose is equal to 0.71 μg/kg [14] and the lowest one mentioned in the research. This critical effect was probably selected due to skeletal muscle tremor being the most frequently mentioned by experts as a critical adverse effect under short-term oral administration of zilpaterol [15–18].

An acceptable daily intake (ADI) was established as equal to 0–0.04 μg/kg of body weight by applying a default uncertainly factor of 10 (the results extrapolated on the most sensitive people) and an additional uncertainty factor of 2 for the use of a LOAEL instead of a NOAEL. Still, there are no data on applying an uncertainty factor associated with the extrapolation of the results obtained under short-term exposure on long-term exposure scenarios despite absence of no-effect exposure levels established for critical effects. Besides, an uncertainty factor of 2, which is used to account for using LOAEL instead of NOAEL, hardly seems sufficient.

At the same time, it is advisable to bear in mind that zilpaterol, given its elimination half-life from animals under oral administration varying between 3.69 to 4.81 hours [5], can still remain in the body in some quantities during a day. This may lead to up to 2 % of administered zilpaterol dose remaining in the body. Given that, we can conclude that daily consumption of meat containing zilpaterol in residual quantities can be considered a chronic exposure factor.

Wide prevalence of cardiovascular diseases among adults as well as risk factors that cause them is an additional factor, which, in our opinion, should be considered. Therefore, potential adverse effects on the cardiovascular system are no less important and we should note that they have been reliably detected both in acute and chronic experiments, anabolic impact of zilpaterol taken into account.

Since we were not able to find any relevant data on effects produced by zilpaterol on the cardiovascular system, we resorted to mathematical modeling to predict risks caused by functional disorders of the CVS under exposure to this additive. We created a mathematical model that described a health risk in dynamic under two scenarios involving daily intake of meat with zilpaterol contents being equal to an MPL suggested by the Joint FAO/WHO Expert Committee (0.0005 mg/kg) and the limit of detection (LoD) for it in meat products (0.0001 mg/kg) [19]. Prediction relied on using an evolution model that described accumulating risks of the CVS functional disorders in accordance with the methodical guidelines by the Eurasian Economic Commission[2].

We applied a recurrent relation to calculate accumulation of risks of functional disorders:

$$R_{t+1} = R_t + (\alpha \cdot R_t + \beta \cdot D)C \qquad (1)$$

with a preset initial risk level R_0, where

R_{t+1} is a risk of disorders at a time moment t+1;

R_t is a risk of disorders at a time moment t;

α is a risk evolution factor due to natural causes;

β is a factor of zilpaterol effects;

C is a time empirical coefficient ($C = 0.00274$ for daily averaging);

D is a zilpaterol dose, mg/kg.

[2] MR 2.1.10.0062-12. Kolichestvennaya otsenka nekantserogennogo riska pri vozdeistvii khimicheskikh veshchestv na osnove postroeniya evolyutsionnykh modelei: metodicheskie rekomendatsii. [MR 2.1.10.0062-12. Quantification of non-carcinogenic risk under exposure to chemicals based on evolution models: methodical guidelines]. Moscow, Rospotrebnadzor's Federal Center for Hygiene and Epidemiology, 2012, 36 p. (in Russian).

The factor of zilpaterol effects was established based on a model that described an 'exposure – likelihood of effect' relationship and was created considering data on influence exerted by zilpaterol on changes in likelihood of an effect:

$$P = b_0 + b_1 \cdot D \quad (2)$$

where P is likelihood of effect, D is a zilpaterol dose [mg/kg]; $b_0 = 0.007$, $b_1 = 185.2$ are model parameters.

Given the established relationship, the coefficient β value in the equation (1) is determined as per the following formula:

$$\beta = g \cdot b_1 \quad (3)$$

where β is the coefficient that describes zilpaterol effect on a risk of this effect;

$g = 0.05$ is a coefficient that shows severity of the effect;

$b_1 = 185.2$ is a coefficient that shows influence exerted by zilpaterol on changes in likelihood of an effect.

The applied values of the coefficient considered, the ultimate value of the coefficient β was equal to 9.26.

Ultimately, the recurrent equation to calculate a risk of an effect occurring under exposure to zilpaterol at the lower boundary of the estimated level was given as follows (4):

$$R_{t+1} = R_t + \left(0.0835 \cdot R_t + 9.29 \cdot D\right) \cdot 0.00274 \quad (4)$$

with the initial condition $R_t = 1.9 \cdot 10^{-4}$.

This equation was used to build a curve that showed evolution of a health risk caused by functional disorders of the CVS under exposure to the zilpaterol dose D (calculated risk) and an adjoining curve built without zilpaterol effects taken into account ($D = 0$) (background risk). An additional risk (ΔR_t) is determined for each time moment as a difference between the background and calculated risk levels.

An acceptable risk level was estimated as per the value of the reduced risk index according to the risk assessment methodology accepted by the Eurasian Economic Commission [20]. Meat consumption was estimated based on statistical data collected in the Russian Federation[3].

The modeling made it possible to establish that consumption of meat that contained zilpaterol in quantities equal to the MPL recommended by the Codex Alimentarius Commission created unacceptable health risks already by 35 years of age. At the same time, if zilpaterol was contained in consumed meat in a quantity equal to the lowest limit of its analytical detection in meat, a health risk would become unacceptable by 55 years of age (Table 3).

Therefore, daily zilpaterol intake with meat in quantities even equal to the level of sensitivity for the method of detection can create impermissible health risks.

Conclusion. The maximum permissible level of zilpaterol in meat suggested by the JECFA, which is equal to 0.5 μg/kg, is based on the ADI established on the basis of the LOAEL under short-term exposure. It does not allow for chronic effects on the nervous and cardiovascular systems; when modifying factors are used, extrapolation of the results obtained by exploring short-term effects on long-tern exposure is not considered.

This allows us to conclude that the MPL for zilpaterol in meat suggested by the JEFCA are not well-grounded. Besides, our modeling of health risks associated with adverse effects caused by consumption with meat with zilpaterol in residual quantities revealed that

[3] Ratsion pitaniya naseleniya 2013: statisticheskii sbornik [Population diets in 2013: statistical data collection]. *Rosstat*. Moscow, IITs 'Statistika Rossii', 2016, 220 p. (in Russian).

Table 3

The reduced health risk index caused by functional disorders of the cardiovascular system under exposure to zilpaterol under different scenarios of its intake with meat

Age, years	The risk index when zilpaterol is contained in consumed meat in quantity equal to suggested MPL	The risk index when zilpaterol is contained in consumed meat in quantity equal to lowest LoD
5	0.0014	0.0003
10	0.0046	0.0009
15	0.0092	0.0018
20	0.0147	0.0029
25	0.0231	0.0046
30	0.0358	0.0072
35	**0.0550***	0.0110
40	**0.0842**	0.0168
45	**0.1285**	0.0257
50	**0.1959**	0.0392
55	**0.2987**	**0.0597**
60	**0.4561**	**0.0912**
65	**0.6984**	**0.1397**

Note: * risk levels higher than acceptable ones are given in bold.

impermissible health risks could occur even under exposure to this food additive in a concentration close to the level of sensitivity for the method of detection.

Consequently, it seems rather premature to accept the maximum permissible level for zilpaterol in meat that is being suggested at present. It is recommended to cut its level down to the lowest limit of detection.

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