COMMONWEALTH OF AUSTRALIA

THE PATENTS ACT 1952-1969

CONVENTION APPLICATION FOR A PATENT

We, SPOFA Spojene podniky pro zdravotnickou vyrobu,
of Praha, Czechoslovakia.

hereby apply for the grant of a Patent for an invention
entitled: "2-FORMYLQUINOXALINE-1,4-DIOXIDE"

which is described in the accompanying complete specification.
This application is a Convention application and is based on the
application(s) numbered: PV 5118-77

for a patent or similar protection made in Czechoslovakia

on 2nd August, 1977

Our address for service is care of GRIFFITH, HASSEL & FRAZER,
Patent Attorneys, of 323 Castlereagh Street, Sydney 2000, in the
State of New South Wales, Commonwealth of Australia.

DATED this 26th day of June, 1978

SPOFA Spojene podniky pro zdravotnickou vyrobu
By their Patent Attorneys:

________________________
of GRIFFITH, HASSEL & FRAZER
Fellows, Institute of Patent
Attorneys of Australia
DECLARATION FORM FOR COMPANY APPLICATIONS, AUSTRALIA

Form 7, 8.

Applicant: Spol' pardobin prostredkov (Incorporated by Commercial Code of Austria)

DEPARTMENT OF COMMERCE

PATENT ACT 1972-69

DECLARATION IN SUPPORT OF AN APPLICATION FOR A PATENT

In support of the Application made by Spofa spol' pentinu pro
zdravotnickou vyrubu.

for a patent for an invention entitled: "2-FORMYLQUINOXALINE-1,4-DIOXIDE"

I, Michal Ivanko

of the Applicant's

address, do solemnly and sincerely declare as follows:

1. I am authorized by the applicant for the patent to make this Declaration on his behalf.

2. Jaromir Habsky, Vladimir Lupinek, Milan Sova, Bohumil Sevcik and Jiri Broz,

   No. 24 Zdanova, Praha 6; No. 17 Viklefova, Praha 3;

   (Full Name of Inventor)

   of

   No. 36/47 Vrhlacceho, Praha 5; No. 79 Pohori, Jilove u

   Prahy and No. 420 Rudnych dolu, Jilove u Prahy,

   all Czechoslovakia respectively.

   Before the actual inventor(s) of the invention and the applicant is the assignee of the said inventor(s).

3. The basic application(s) as defined by Section 141 of the Act, was made in

   Czechoslovakia

   on the 2nd

   day of

   August, 1977

   by the inventors.

   (Name of Applicant for Basic Application)

4. The basic application(s) referred to in paragraph 3 of this Declaration was the first application(s) made in a Convention country in respect of the invention, the subject of the application.

Declared at Praha, Czechoslovakia

this 12th

day of June, 1978.

To:

The Commissioner of Patents,

CANBERRA.

NOTE: Initial all declarations and attachments.

No other names and no application.

For each application, attach a copy of the basic application.

For applications for a patent in Austria, attach a copy of the basic application.

For applications for a patent in any other Convention country, attach a copy of the basic application.

For later applications by an assignee, and not for a patent that does not fall within the scope of the 4

applicants and is connected by the third applicant to make an application for a further invention (Form 8-1),

and not the Commissioner, has power to permit the insertion from 3 (Form 8-2).

GRIFFITH, HASSEL & PFAALZER,

Box 2133, G.P.C.,

SYDNEY 2001, AUSTRALIA.
2-Formylquinoxaline-1,4-dioxide cyanoacetylhydrazones of the general formula I

\[ \text{CH}_2=\text{N-NH-COCH}_2\text{CN} \]

in which \( R^1 \) is a hydrogen atom or a lower alkyl group having 1 to 4 carbon atoms.
The following statement is a full description of this invention, with the best method of performing it known to me/us:

"2-FORMYLQUINOXALINE-1,4-DIOXIDE"
The present invention provides novel 2-formylquinoxaline-1,4-dioxide cyanoacetylhydrazones of the general formula I

\[
\begin{array}{c}
\text{CH}=\text{N}-\text{NH-} \text{COCH}_2\text{CN} \\
\text{O} \\
\text{N} \\
\text{N} \\
\text{R}^1
\end{array}
\]

in which \( R^1 \) is a hydrogen atom or a lower alkyl group having 1 to 4 carbon atoms. The invention also provides processes for the preparation of these compounds as well as compositions containing them.

Certain acylhydrazones of the parent 2-formylquinoxaline-1,4-dioxide and its derivatives are already known /GB-PS 2 102 770, US-PS 3 433 871, CS-author certificate ... = CS-PA 1788-75/. Surprisingly it has now been found that cyanoacetylhydrazones of 2-formylquinoxaline-1,4-dioxide, which are all novel, hitherto unknown substances, possess in addition to valuable antimicrobial action both on gram-positive and grammnegative microorganisms - also markedly pronounced anticoccidial properties, show important promotory effect on the growth of farm animals, and favourably influence their general health status.

In comparison with the known nonantibiotic animal growth promotants routinely employed in the practice, the novel compounds of the invention have significant advantage in their substantially diminished toxicity. Thus, a comparative
study of the toxic properties of the 2-formylquinoxaline-1,4-dioxide cyanoacetylhydrazone of the invention /compound A/ and, as the reference substance, the known 2-formylquinoxaline-1,4-dioxide methoxycarbonylhydrazone /compound B/ was made in a series of parallel experiments performed on male rats of 190 to 240 g body weight grouped in groups of 10 animals each. The compounds under test were administered orally in the form of 2.5% suspension in an aqueous arabic gum solution. Single doses as high as 500 mg/kg /2 ml. of the suspension/ were given to the animals twice a day, also 1 g/kg daily, always at 9 and 13 o'clock over five successive days, i.e., altogether ter doses of either compound were administered. The subsequent Table I indicates the results of observing the mortality rate of the individual animals upon administering the respective overall dose of the compounds under study.

**TABLE I**

Mortality of test animals following the indicated dosage

<table>
<thead>
<tr>
<th>Control group Rat No.</th>
<th>/Reference/ Compound B Overall dose, g/kg</th>
<th>Test Group Rat No.</th>
<th>Compound A Overall dose, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>5.0</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>
As evident from the above Table, the tested compound A of the invention is significantly less toxic than the reference compound B: all the animals of the control group died whereas, on the contrary, only two perished out of the ten in the test group treated with the compound of the invention and the rest of these animals survived. The animals were observed over additional 5 days following after the day of administration of the last dose.  

An attempt was made to determine, using the standard testing technique, the LD$_{50}$ value of compound A on female Wistar rats of 140 to 160 g body weight, grouped again in groups of ten animals each. The tested compound A was administered orally, in a single dose, in the form of a 20% suspension in Dorfman medium. This attempt to estimate the acute toxicity, however, failed since it was impossible to increase the administered doses of the tested compound over 5 500 mg/kg which threshold dose was still sublethal. Consequently, for compound A, LD$_{50}$ is substantially higher than 5 500 mg/kg orally p.o.

As demonstrated by a series of experiments on broiler chickens, piglets and hogs, the compounds of the invention
markedly stimulate the total performance of farm animals, particularly by increasing their liveweight gains, enhancing the utilization of feed mixtures, reducing the incidence of diarrhoea in piglets, and thus contributing to improvement of their general health status. Also, the compounds of the invention can advantageously be employed, either per se alone or if required in combination with known veterinary medicaments, for the prevention and treatment of salmonelloses and dysenterias in hogs and other farm animals.

The compounds can be administered either per se in pure state or preferably as mixtures with common carriers, excipients and auxiliaries, in dispensing forms accepted for veterinary purposes and animal nutrition, most preferably as concentrates and premixes suitable for the compounding of feed mixtures.

The compounds of the invention, of the general formula I, can be prepared by a series of methods known per se for the production of hydrazones. Thus, a 2-formylquinoxaline-1,4-dioxide of the general formula II

\[
\begin{align*}
\text{O} & \\
\text{CHO} & \\
\text{R}^1 & \text{N} \\
& \text{O}
\end{align*}
\]  

(II)

in which \( R^1 \) has the above defined meaning, or an acetal-like functional derivative thereof, is reacted with cyanoacetyl-hydrazine of the formula
This reaction is conveniently carried out in suitable inert organic or inorganic solvents or diluents or mixtures thereof such as aliphatic or aromatic hydrocarbons or their halogenated derivatives, e.g. benzene, toluene, chlorobenzene, dichloromethane or chloroform, carboxylic acids such as, e.g. formic or acetic acid, or aliphatic alcohols, e.g. methanol or ethanol, if required with addition of an organic or inorganic acid such as, e.g. formic, acetic, hydrochloric or p-toluene-sulphonic acid and the like or mixtures thereof. The reaction proceeds with a sufficient reaction rate even at ambient temperature and can be conducted either at lowered or elevated temperatures, suitably ranging from approximately 0°C to the boiling temperature of the reaction mixture. The reaction time required is dependent on the nature of the starting materials used as well as on the reaction medium and temperature chosen, and generally varies from several minutes to approximately 24 hours. The reaction yields are as a rule nearly quantitative.

Another convenient process for the production of the compounds of the invention resides in that a 2-formylquinoxaline-1,4-dioxide hydrazone of the general formula III

\[
\text{H}_2\text{N}-\text{NH-COCH}_2\text{CN}
\]

Another convenient process for the production of the compounds of the invention resides in that a 2-formylquinoxaline-1,4-dioxide hydrazone of the general formula III

\[
\text{III}
\]

\[
\text{III}
\]
in which R has the herin before defined meaning, is reacted
with a suitable reactive derivative of cyanoacetic acid,
preferably with cyanoacetyl chloride or a cyanoacetic acid
ester, particularly a halogenated phenyl cyanoacetate, in
an inert organic or inorganic solvent or diluent or in a
mixture thereof, at a temperature from -20 to -100°C.

The starting materials of the general formula III are
available by reacting the respective parent 2-formylquinoxaline-
1,4-dioxide with hydrazine. Said reactive derivatives of
cyanoacetic acid are known from the literature or can also
be prepared by per se known methods.

Still another process for the production of the compounds
of the invention consists in reacting the respective 2-formyl-
quinoxaline-1,4-dioxide of the general formula II hereinbefore
first with chloroacetylhydrazine of the formula

\[ H_2N-NH-COCH_2Cl \]

/J.S. Pizey and R.L. Wain, J. Sci.Agr. 10, 577, 1959/ and
subsequently with an alkali metal cyanide. Again, these two
reactions are carried out by methods known per se.

A further process for the production of the compounds
of the invention involves reacting the 2-formylquinoxaline-
1,4-dioxide hydrazone of the general formula III hereinbefore
successively with chloroacetyl chloride in the presence of
an organic or inorganic base, preferably a tertiary amine,
in a suitable inert organic or inorganic medium, and thereafter
with an alkali metal cyanide.

Still alternatively, it is also possible to prepare the compounds of the invention by the reaction of a 2-formylquinoxaline-1,4-dioxide oxime of the general formula IV

![Diagram of formula IV](image)

in which $R^1$ has the meaning as defined hereinbefore, with cyanoacetylhydrazine in an aqueous inorganic acid solution, preferably in 30 to 98% sulphuric acid, at an elevated temperature, conveniently from 20 to 100°C.

The following examples illustrate the invention in detail but are in no respect limitative as to the scope thereof. The melting points below indicated were determined on Kofler block and are uncorrected.

**EXAMPLE 1**

2-Formylquinoxaline-1,4-dioxide cyanoacetylhydrazone

**METHOD A**

A solution of 15.2 g/0.08 mole of 2-formylquinoxaline-1,4-dioxide in 150 ml of ethanol is treated with 7.9 g /0.08 mole/ of cyanoacetylhydrazine /melting at 105-107°C/, the mixture is stirred 4 hours at room temperature and the precipitated crude product is sucked off. The yield is 18.4 g /85% of theory/ of the crude title compound. Upon crystallization from
dimethylformamide, yellow crystals melting at 250 - 260°C /with decomposition/ are obtained.

METHOD B

A mixture of 23.6 g /0.1 mole/ of 2-formylquinoxaline-1,4-dioxide dimethylacetal and 500 ml of methanol is treated with 200 ml of concentrated hydrochloric acid and the whole is agitated until complete dissolution. After that, 10.5 g /0.105 mole/ of cyanoacetylhydrazine is added and the reaction mixture is stirred 3 hours at room temperature. The precipitated crude product is sucked off, washed with methanol and dried. The yield is 24.4 g /90% of theory/ of yellow substance melting at 255 - 260°C /decomposition/.

EXAMPLE 2

2-Formyl-3-methylquinoxaline-1,4-dioxide cyanoacetylhydrazone

A suspension of 20.4 g /0.1 mole/ of 2-formyl-3-methylquinoxaline-1,4-dioxide /m.p. 180-182°C/ and 9.9 g /0.1 mole/ of cyanoacetylhydrazine in 200 ml of ethanol is stirred 4 hours at room temperature, and the separated product is sucked off and washed with ethanol. The yield is 22.2 g /78% of theory/ of the crude substance. Upon crystallization from dimethylformamide, yellow crystals melting at 255 - 260°C /decomposition/ are obtained.
EXAMPLE 3

The commercial standard complete feed mixture BR-1 for feeding broiler chickens was enriched by admixing to it, in a quantity of 1% by wt., a feed supplement containing specifically active ingredients in the following ratio:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Formylquinoxaline-1,4-dioxide</td>
<td>5000</td>
</tr>
<tr>
<td>cyanoacetylsalicylic acid /compound I/</td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>I.U.</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>I.U.</td>
</tr>
<tr>
<td>Vitamin B₂</td>
<td>mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>mg</td>
</tr>
<tr>
<td>Vitamin K₃</td>
<td>mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>mg</td>
</tr>
<tr>
<td>Methionin</td>
<td>mg</td>
</tr>
<tr>
<td>Amprol - ethopabate 25% premix</td>
<td>mg</td>
</tr>
<tr>
<td>Ethoxyquine /antioxidant/</td>
<td>mg</td>
</tr>
</tbody>
</table>

Upon feeding the supplemented mixture of Tetra SL chickens in the standard feeding test, an increase of the weight gain by 12.6% in comparison to the control group was obtained, and the feed conversion was enhanced by 7.0% over the controls.

In a feed experiment performed on Ross-1 broiler chickens, the above compound I was admixed to the same standard complete feed mixture BR-1 in doses of 10, 20, 50 and 100 mg/kg of the mixture. In the course of first 4 weeks of the feeding experiment, the weight gains improved, respectively, by 5.6%,
8.5% statistically significant/, 8.6% statistically significant/, and 5.5% in comparison with the control group. Simultaneously, the feed conversion improved by a margin ranging from 0.9 to 5.3%.

**EXAMPLE 4**

The commercial standard complete feed mixture COS-2 for feeding early-weaned piglets was enriched by admixing to it, again in the quantity of 1% by wt., a feed supplement containing specifically active ingredients in the following ratio:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound I</td>
<td>mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>I.U.</td>
</tr>
<tr>
<td>Vitamin (D_3)</td>
<td>I.U.</td>
</tr>
<tr>
<td>Vitamin (B_2)</td>
<td>mg</td>
</tr>
<tr>
<td>Vitamin (B_6)</td>
<td>mg</td>
</tr>
<tr>
<td>Vitamin (K_3)</td>
<td>mg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>mg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>mg</td>
</tr>
<tr>
<td>Calcium pantothenate</td>
<td>mg</td>
</tr>
<tr>
<td>Lysine</td>
<td>mg</td>
</tr>
<tr>
<td>Ethoxyquine</td>
<td>mg</td>
</tr>
</tbody>
</table>

Upon feeding the supplemented mixture to early-weaned piglets over 28 days, statistically significant improvement
of daily weight gains by 44.1% in comparison with the control group was observed and the feed conversion improved by 20.9% in the first experiment. Similarly, in the other analogous parallel trial, the corresponding weight increase and feed conversion improvement values over the controls were 39.9 and 33.7%, respectively. Also, in the group fed the supplemented mixture containing the above compound of the invention, not a single case of diarrhoea in piglets following their early weaning occurred and, in either series of the experiments reported, high utility performance of the treated animals was achieved.
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. 2-Formylquinoxaline-1,4-dioxide cyanoacetylhydrazone of the general formula I

![Chemical Structure I](image)

in which \( R^1 \) is a hydrogen atom or a lower alkyl group having 1 to 4 carbon atoms.

2. 2-Formylquinoxaline-1,4-dioxide cyanoacetylhydrazone.

3. 2-Formyl-3-methylquinoxaline-1,4-dioxide cyanoacetylhydrazone.

4. A process for the production of the 2-formylquinoxaline-1,4-dioxide cyanoacetylhydrazones of the above general formula I, as claimed in claim 1, characterized in that a 2-formylquinoxaline-1,4-dioxide of the general formula II

![Chemical Structure II](image)

in which \( R^1 \) has the hereinbefore defined meaning, or an
acetal-like functional derivative thereof is reacted with cyanoacetylhydrazine of the formula

\[ H_2N-NH-COCH_2CN \]

in an inert organic or inorganic solvent or diluent or in a mixture thereof, at a temperature from 0°C to the boiling temperature of the reaction mixture.

5. A process for the production of the compounds of claim 1, characterized in that a 2-formylquinoxaline-1,4-dioxide hydrazone of the general formula III

\[ \text{III} \]

in which \( R^1 \) has the hereinbefore defined meaning, is reacted with a reactive derivative of cyanoacetic acid in an inert organic or inorganic solvent or diluent or in a mixture thereof, at a temperature from -20 to -100°C.

6. A process of claim 5, characterized in that cyanoacetyl chloride or a cyanoacetic acid ester is used as said reactive derivative of cyanoacetic acid.
7. A process for the production of the compounds of claim 1, characterized in that a 2-formylquinoxaline-1,4-dioxide of the general formula II hereinbefore is reacted with chloroacetylhydrazine of the formula

\[ \text{H}_2\text{N-NH-COCH}_2\text{Cl} \]

in an inert organic or inorganic solvent or diluent or in a mixture thereof, and thereafter with an alkali metal cyanide.

8. A process for the production of the compounds of claim 1, characterized in that a 2-formylquinoxaline-1,4-dioxide hydrazone of the general formula III hereinbefore is reacted with chloroacetylchloride in the presence of an organic or inorganic base in an inert organic or inorganic solvent or diluent or in a mixture thereof, and thereafter with an alkali metal cyanide.

9. A process of claim 8, characterized in that an alkali metal carbonate or hydrogencarbonate or a tertiary amine such as pyridine or triethylamine is used as said base.

10. A process for the production of the compounds of claim 1, characterized in that a 2-formylquinoxaline-1,4-dioxide oxime of the general formula IV

\[ (IV) \]
in which $R^1$ has the hereinbefore defined meaning, is reacted with cyanoacetyldrazine in an aqueous inorganic acid solution, preferably in 30 to 98% sulphuric acid, at a temperature from 20 to 100°C.

11. Compositions for purposes of veterinary medicine or animal nutrition, containing at least one compound of claim 1 as the active principle together with known carriers, excipients or auxiliaries.

Dated this 26th day of June, 1978.