GRAS Notification for Use of Berberine as a Preservative in Feed for Broiler Chickens

SUBMITTED BY:

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SUBMITTED TO:

U.S. Food and Drug Administration
Center for Veterinary Medicine
Office of Surveillance and Compliance
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January 25, 2021

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List of Acronyms

°C	Degrees Celsius
%	Percent
µg	Microgram
AAFCO	Association of American Food Control Officials
ADME	Absorption, distribution, metabolism, and excretion
AT	Adenine Thymine
AUC	Area under the curve
BCD	Berberine chloride dihydrate
BHC	Benzene hexachloride
BN	Batch number
bw	Body weight
CAR	Constitutive Androstane Receptor
CASRN	Chemical Abstracts Service Registry Number
CFDA	China Food and Drug Administration
CFR	Code of Federal Regulations
cfu	Colony-forming unit
cGMP	Current Good Manufacturing Practice
COA	Certificate of analysis
CVM	Center for Veterinary Medicine
DHHS	U.S. Department of Health and Human Services
DLC	Dioxin like compounds
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
EDB	Ethylene dibromide
EDI	Estimated Daily Intake
EFSA	European Food Safety Authority
FARE®	Foods Analysis and Residue Evaluation Program
FCR	Feed consumption ratio
FDA	U.S. Food and Drug Administration
FRC	Fibrous root of *Rhizoma Coptidis*
FSANZ	Food Standards Australia New Zealand
FNDSS	Food and Nutrient Database for Dietary Studies
FPED	Food Patterns Equivalents Database
FPEID	Food Patterns Equivalents Ingredients Database
FSIS	Food Safety and Inspection Service
g	Gram
GD	Gestational days
GFI	Guidance for Industry
GLP	Good Laboratory Practice

GRAS	Generally Recognized As Safe
HPLC	High-performance liquid chromatography
HPTLC	High-performance thin-layer chromatography
IARC	International Agency for Research on Cancer
i.p.	Intraperitoneal
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOQ	Limit of quantification
LPS	Lipopolysaccharide
kg	Kilogram
MIC	Minimum inhibitory concentration
mo	Month
MOE	Margin of exposure
mg	Milligram
MTL	Maximum tolerance level
ng	Nanogram
NLT	No less than
NMT	No more than
NCHS	National Center for Health Statistics
NTP	National Toxicology Program
NOAEL	No observed adverse effect level
NHANES	National Health and Nutrition Examination Survey
NRC	National Research Council
OECD	Organisation for Economic Co-operation and Development
PCB	Polychlorinated biphenyls
ppm	Parts per million
QC	Quality control
TEQ	Toxicity Equivalent
TTB	Department of Treasury Alcohol and Tobacco Tax and Trade Bureau
USDA	United States Department of Agriculture
WHO	World Health Organization
WWEIA	What We Eat in America

Part 1: Signed Statements and Certification (21 CFR § 570.225)

IRP Health PTY LTD, also known as IRP Health Inc. ("IRP Health") submits to the U.S. Food and Drug Administration (FDA) this generally recognized as safe (GRAS) notice in accordance with the 21 CFR part 570, subpart E.

Name and Address of Notifier

IRP Health PTY LTD
C/- McBain McCartin & Co Business Services Pty Ltd
Level 1, 123 Whitehorse Road
Balwyn VIC, Australia 3103

Name of GRAS Substance

The substance that is the subject of this GRAS notice is berberine, with a purity of NLT 97.0% berberine (anhydrous basis). The chemical name is 5,6-Dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-α] quinolizinium chloride hydrate.

Intended Use, Target Animal and Consumer Exposure

IRP Health's berberine is intended for use as a preservative in feed for broiler chickens. Berberine will be added to feed at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold. Results from a poultry feeding study showed that at the proposed use level, berberine residues in chicken tissues were not detected at the limit of detection (LOD) of 2 ng/g.

Basis for Conclusion of GRAS Status

IRP Health's conclusion of GRAS status for the intended use of berberine as a preservative in feed for broiler chickens is based on scientific procedures in accordance with 21 CFR 570.30(a) and (b)

Pre-Market Approval Exclusion Claim

Use of IRP Health's berberine is not subject to the pre-market approval requirements of the Federal Food, Drug, and Cosmetic Act because IRP Health has concluded that such use is generally recognized as safe (GRAS) based on scientific procedures.

Availability of Information

The data and information that serve as the basis for this GRAS conclusion, as well as the information that has become available since the GRAS conclusion, will be sent to the FDA upon request, or are available for the FDA's review and copying during customary business hours at the office of Riëtte van Laack, Hyman, Phelps & McNamara, P.C., 700 Thirteenth Street, N.W., Suite 1200, Washington, DC 20005.

Exemptions from Disclosure

It is our view that none of the data and information in Parts 2 through 7 of the GRAS notice are exempt from disclosure under the Freedom of Information Act (FOIA).

Certification Statement

On behalf of IRP Health, I hereby certify that, to the best of my knowledge, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable, as well as favorable information, known to me and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

(b) (6)

January 25, 2021

Name: Miguel Motta
Title: President and Chief Executive Officer
Company: IRP Health PTY LTD

Date

Part 2. Identity, Method of Manufacture, Specifications, and Technical Effect (21 CFR § 570.230)

Identity

The chemical name of substance of this review is 5,6-Dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-α] quinolizinium chloride hydrate.

Common name: Berberine, berberine chloride

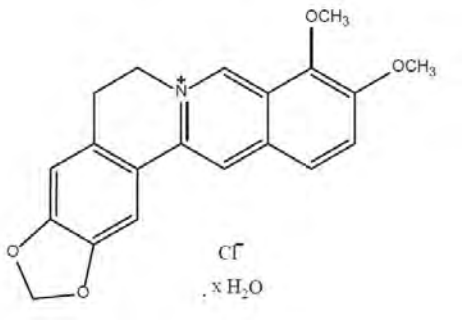
C.A.S. number: 633-65-8 (Anhydrous)

Chemical formula: $C_{20}H_{18}ClNO_4 \cdot xH_2O$

Molecular weight: 371.81 (Anhydrous)

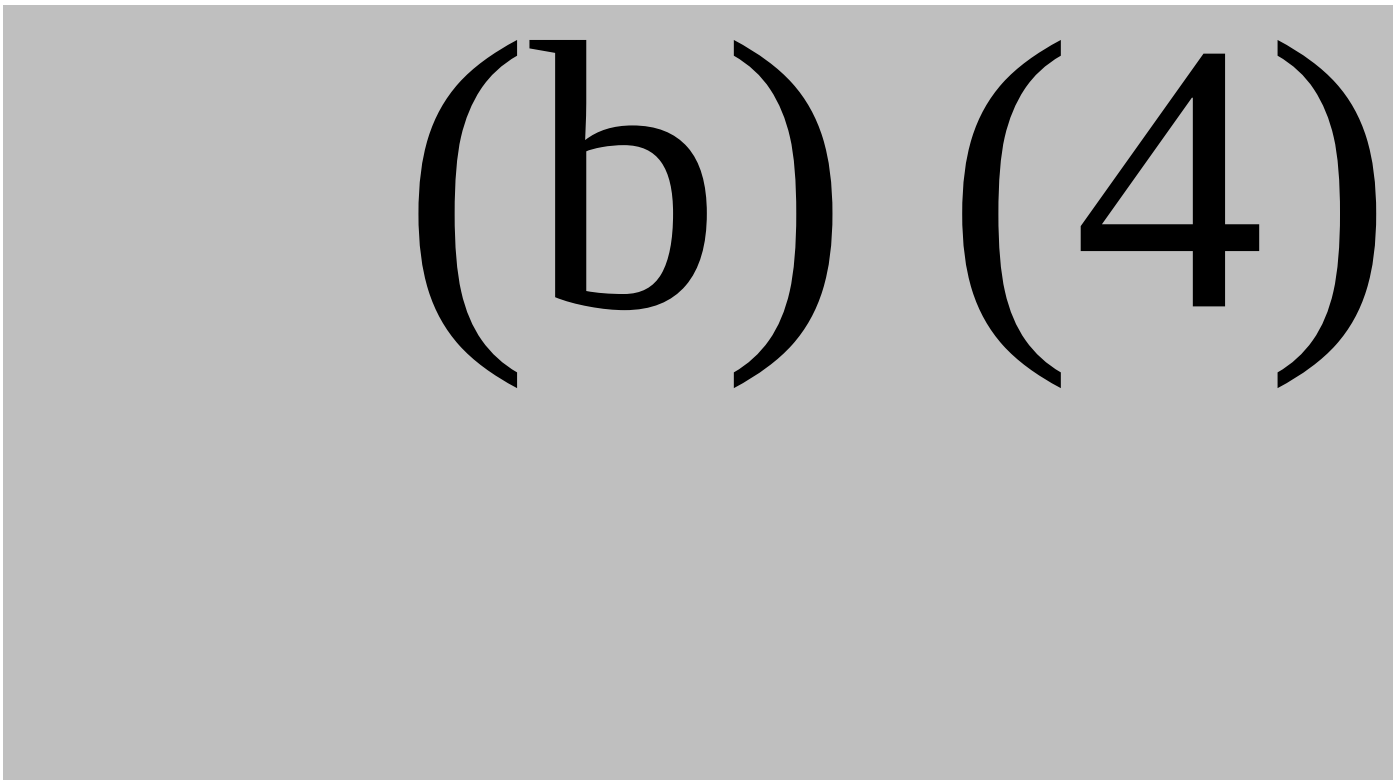
The structure of berberine is shown in Figure 1.

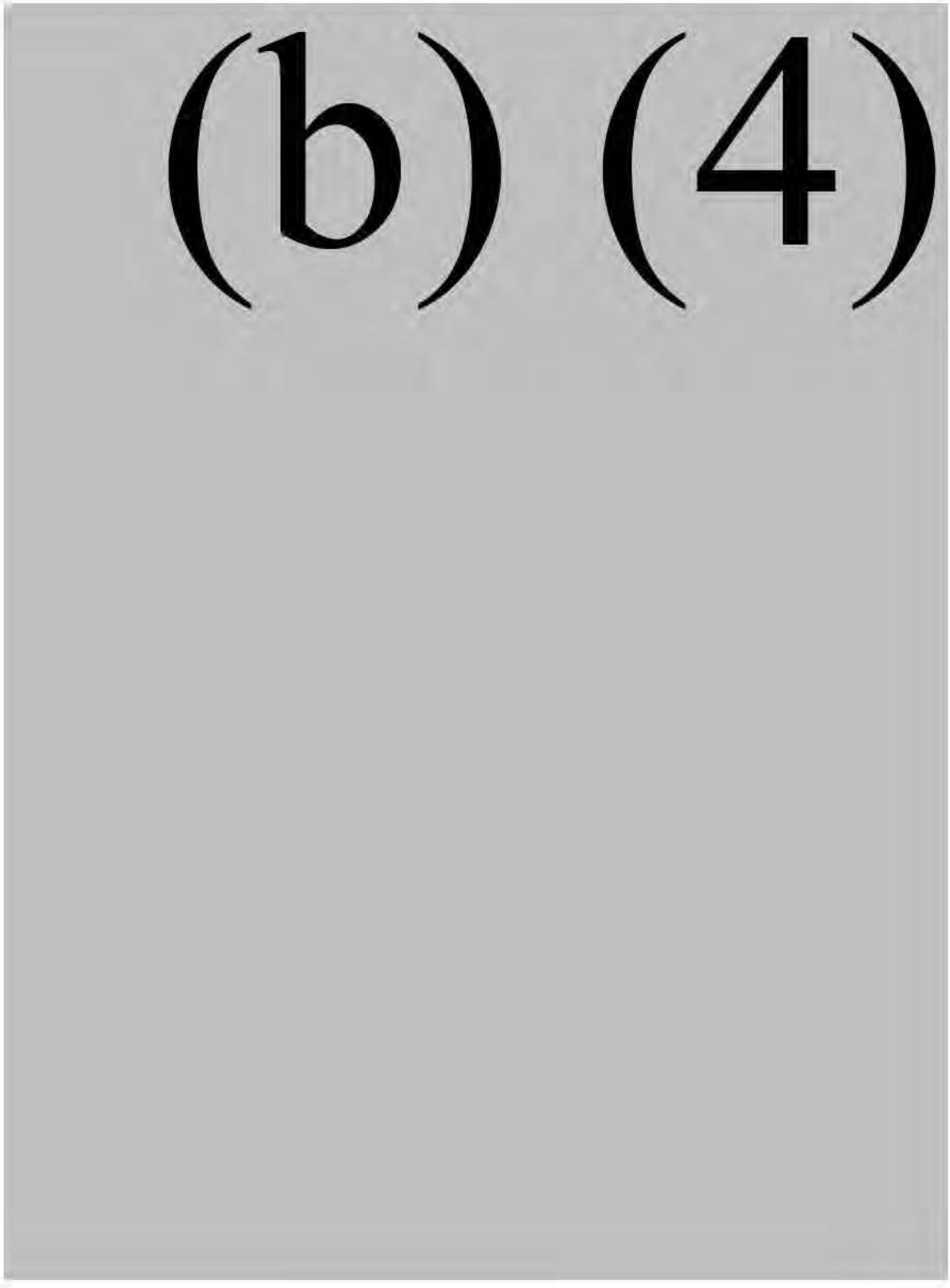
Figure 1. Structure of berberine



Method of Manufacture

(b) (4)


(b) (4)


(b) (4)

Specifications for the Substance

IRP Health's extracted berberine product, the subject of this GRAS notification, is a yellow fine powder, with a purity of NLT 97.0% berberine (anhydrous basis). Specifications for the substance are summarized in **Table 1**.

The specification limits of heavy metals in the extracted berberine product, including lead (no more than (NMT) 3.0 mg/kg)), arsenic (NMT 2.0 mg/kg), cadmium (NMT 0.2 mg/kg) and mercury (NMT 0.1 mg/kg) are well below the Association of American Food Control Officials (AAFCO) maximum tolerance levels (MTLs) for these metals in complete feed (i.e., 30 mg/kg (lead), 50 mg/kg (arsenic), 0.5 mg/kg (cadmium), and 2 mg/kg (mercury)) (AAFCO, 2019). The metal specification limits in the extracted berberine product are also well below the National Research Council (NRC) MTLs for complete feed (i.e. 10 mg/kg (lead), 30 mg/kg (arsenic), 10 mg/kg (cadmium) and 0.2 mg/kg (mercury)) (NRC, 2005)[1]. Furthermore, the intended use level of the extracted berberine is 0.030g/kg of feed (or 30 ppm). As such, the corresponding limits in the complete feed will only be 0.003% of the specification limits in the extracted berberine product.

Solvent: (b) (4) is the only organic solvent used in the extraction process. The specification limit is less than (b) (4) The intended use level of the extracted berberine is 0.030g/kg of feed (or 30 ppm). As such, the (b) (4) limit in complete feed will be <0.15 ppm.

Pesticides specification limits are in compliance with USP 42 <561>.

Microbial limits conform to USP 40 <61> and USP 40 <62>.

[1] Values cited are those for the most sensitive animal species in NRC's 2005 edition of "Mineral Tolerance of Domestic Animals)

Mycotoxin specifications for berberine is not needed since mycotoxin production is closely related with mold number and berberine inhibits mold growth, see intended use and efficacy data below.

Table 1. Specifications

Specifications		**Method of Analysis**
Appearance	Yellow fine powder with characteristic odor and taste	
Identification	Should be identical to reference standard sample	HPTLC; See description in **Appendix B**
Purity		
Assay	$C_{20}H_{18}ClNO_4$ extract NLT 97.0%	Calculated on the anhydrous basis. See description in **Appendix B**
Sieve analysis	100 % through 80 mesh	USP40 <786>
(b) (4)	(b) (4)	(b) (4)
Total Ash	NMT 0.5%	Eur.Ph.7.0 [2.4.16]
Lead (Pb)	NMT 3.0 mg/kg	Eur.Ph.7.0 <2.2.58> ICP-MS
Arsenic (As)	NMT 2.0 mg/kg	Eur.Ph.7.0 <2.2.58> ICP-MS
Cadmium (Cd)	NMT 0.2 mg/kg	Eur.Ph.7.0 <2.2.58> ICP-MS
Mercury (Hg)	NMT 0.1 mg/kg	Eur.Ph.7.0 <2.2.58> ICP-MS
(b) (4)	(b) (4)	(b) (4)
Pesticides Residue	Conform to USP	USP42 <561>
Microbial limits		
Total bacteria count	NMT 1000 cfu/g	USP40 <61>
Total yeast & mold	NMT 100 cfu/g	USP40 <61>
E. coli	Negative	USP40 <62>
Salmonella	Negative	USP40 <62>

The results of analyses for three non-consecutive batches demonstrating compliance with specifications are shown in **Table 2.** Pesticides analytical data are provided in **Appendix C.**

Table 2. Batch Analyses from Non-Consecutive Lots

Specifications		BN CHL-A-910527	BN CYSXBJ-C-A091253	BN CYSXBJ-C-A091398
Appearance	Yellow fine powder with characteristic odor and taste	A yellow fine powder with characteristic odor and taste		
Identification	Should be identical to reference standard sample	Identical	Identical	Identical
Purity				
Assay	$C_{20}H_{18}ClNO_4$ extract should be NLT 97.0%	(b) (4)	(b) (4)	(b) (4)
Sieve analysis	100 % through 80 mesh	Conform	Conform	Conform
(b) (4)	(b) (4)	(b) (4)		
Total Ash	NMT 0.5%			
Lead (Pb)	NMT 3.0 mg/kg			
Arsenic (As)	NMT 2.0 mg/kg			
Cadmium (Cd)	NMT 0.2 mg/kg			
Mercury (Hg)	NMT 0.1 mg/kg			
(b) (4)	(b) (4)	Conform	Conform	Conform
Pesticides Residue	Conform to USP42 <561>	Conform		
Microbial limits				
Total bacteria count	NMT 1000 cfu/g	Conform	Conform	Conform
Total yeast & mold	NMT 100 cfu/g	Conform	Conform	Conform
E. coli	Negative	Conform	Conform	Conform
Salmonella	Negative	Conform	Conform	Conform

Dioxin and Polychlorinated Biphenyls

In addition to the parameters assessed as part of the product specifications in **Table 1**, testing of extracted berberine for dioxins and polychlorinated biphenyls (PCBs) are routinely conducted to assure product safety.

Dioxin testing of an extracted berberine sample for 17 dioxin-like compounds (DLC) congeners, including 7 polychlorinated dibenzodioxins compounds and 10 polychlorinated dibenzofuran, were non-detected, except for 4 congeners: 1,2,3,4,6,7,8-HpCDF, OCDF, 1,2,3,4,6,7,8-HpCDD, and OCDD, with reported detected levels of 0.0484, 0.0732, 0.246, and 0.738 ng/kg, respectively. The sample results were compared to levels reported by the FDA's Center for Veterinary Medicine (CVM) in samples of poultry feed. The dioxin levels in the berberine

extract expressed as total equivalent (TEQ, 1998-WHO) (lower bound: 0.003 ng/kg and medium bound: 0.029 ng/kg) are lower than the total 1998-World Health Organization (WHO) TEQ levels reported in the deodorizer distillate and molasses, corn oil, and canola meal samples tested by FDA CVM. The 4 congeners with detection in the berberine sample are also the congeners that have the highest reported mean values for plant-based animal feed in the FDA CVM survey, and the levels in the berberine product are well below the mean levels reported in the CVM samples for these 4 congeners. Details for the sampling results and comparison are provided in **Appendix D**.

The potential dioxin levels in chicken meat and fat that could result from feeding chicken animal feed containing berberine were also estimated and compared to levels detected by the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) and FDA in chicken samples collected in the US. The estimated 1998-WHO total TEQ dioxin levels in chicken associated with the berberine product were comparable to the range of levels reported for chicken food samples in the FDA TDS database and for young chicken in USDA FSIS database. The calculated 2005-WHO total TEQ dioxin level in chicken fat are within the ranges detected in the FDA TDS and USDA FSIS databases. The sampling results and comparisons are detailed in **Appendix D.**

Testing of extracted berberine also shows that PCBs are not detected (limit of quantification, LOQ = 5 ppb), see **Appendix E**. This LOQ is well below the 21CFR 509.30 Temporary tolerances for polychlorinated biphenyls (PCBs) of 0.2 ppm in finished animal feed for food-producing animal.

Stability Data

Stability of Berberine Chloride in Storage

Three batches (BN: Chl20090511, Chl20090609 and Chl20090711) were manufactured and (b) (4). The test items include appearance, (b) (4) and assay using the methods described in product specifications. The batch details and storage conditions are summarized below in **Table 3**.

Table 3. Batch Details and Storage Conditions

Batch number:	Chl20090511, Chl20090609 and Chl20090711		
Batch type:	Production-scale		
Storage conditions:			
Stability Program	*Temperature*	*Humidity*	*Duration*
Long term	25±2°C	60±10%RH	36 months (0, 3, 6, 9,18, 24 and 36)

Berberine chloride is stable under all storage conditions tested. All tested parameters at all time points tested in the studies under the long term conditions (25±2°C /60±10%RH) are within the specifications. Therefore, the stability study data supports a 36 month shelf life when stored at or below 25°C. The stability test data are summarized below in **Table 4**.

Table 4. Berberine Chloride Stability Data (Stability test conditions: 25±2°C /60±10%RH; up to 36 months)

Batch Number: Chl20090511	**Item**	**Acceptable Criteria**	**Test Results**						
			0 mo	**3 mo**	**6 mo**	**9 mo**	**18 mo**	**24 mo**	**36 mo**
	Appearance	Should be a yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder
	(b) (4)	≤ 12.0%	10.62%	(b) (4)					
	Assay	≥ 97.0%	(b) (4)						
Batch Number: Chl20090609	**Item**	**Acceptable Criteria**	**Test Results**						
			0 mo	**3 mo**	**6 mo**	**9 mo**	**18 mo**	**24 mo**	**36 mo**
	Appearance	Should be a yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder
	(b) (4)	≤ 12.0%	(b) (4)	(b) (4)					
	Assay	≥ 97.0%	(b) (4)						
Batch Number: Chl20090711	**Item**	**Acceptable Criteria**	**Test Results**						
			0 mo	**3 mo**	**6 mo**	**9 mo**	**18 mo**	**24 mo**	**36 mo**
	Appearance	Should be a yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder	yellow fine powder
	(b) (4)	≤ 12.0%	8.45%	(b) (4)					
	Assay	≥ 97.0%	97.23%						

Stability Study of Berberine Chloride in Mash and Pellets for Broiler Chickens

In a study conducted by (b) (4), berberine shows good stability in a two-week study of mash and pellets formulated at the intended use concentration for broiler chickens.

Concentrations of berberine in broiler chicken feed after two weeks at room temperature were similar to time zero concentrations, see **Table 5**. The complete study report by (b) (4) is provided in **Appendix F.**

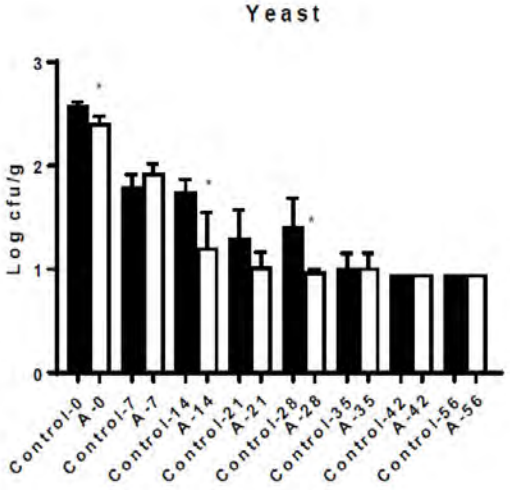
Table 5. Stability of Berberine in Mash and Pellets for Broiler Chickens

Form of Berberine	Time Zero	Day 7	Day 14
Mash	0.03 g/kg	(b) (4)	
Pellet	0.02 g/kg		

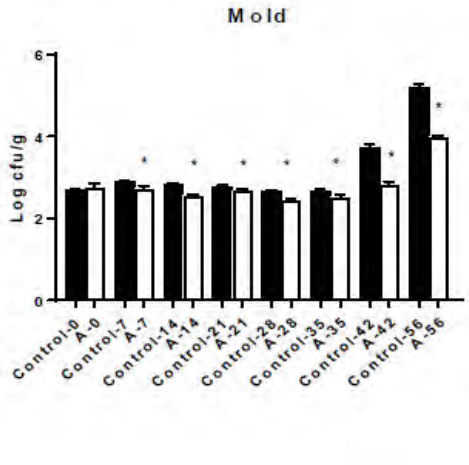
Technical Effect

Berberine is intended to be used as a preservative in the feed of broiler chickens at maximum use level of 0.03 g/kg (30 mg/kg) to preserve feed from yeast and mold. Berberine has demonstrated antimicrobial properties and has been recommended as a plant-derived antimicrobial agent for food preservation (Hintz et al., 2015). A study of the preservative property of berberine in animal feed shows efficacy in controlling yeast and mold at the intended use level of 0.03 g/kg in broiler chicken feed. Data from the study is shown below in Figure 3. The complete study report is located in **Appendix G.**

Figure 3. Concentration of yeast and mold, as log cfu/g, at different time points until day 56



There was a significant difference during the whole study and also at different time points: day 14 and 28. *=p <0.05.



There was a significant difference during the whole study and also at different time points: day 7, 14,21, 28,35,42 and 56*=p <0.05.

Part 3. Target Animal and Human Exposure (21 CFR § 570.235)

Intended Use

Berberine is intended to be used as a preservative in the feed of broiler chickens. The maximum use level is 0.03 g/kg (30 mg/kg) to preserve feed from yeast and mold. Berberine may be combined with other substances permitted for use in broiler chicken feed in the U.S. prior to marketing.

Exposure in Broiler Chickens

Feed intake by poultry varies due to many factors such as size, age, gender, breed, environment, level of egg production, and quality of feed. For a male broiler chicken weighing 3.08 – 3.37 kg, the estimated feed intake is 220.5 g per day, and for a female weighing 2.56 – 2.78 kg, the daily feed intake is estimated to be 200.3 g/day (Rostagno et al, 2017). Based on the proposed inclusion rate of berberine in feed of 0.03 g/kg (or 0.03 mg/g), the daily intake of berberine for broiler chickens are summarized below.

Table 6. Daily Intake of Berberine by Broiler Chickens

Poultry Type	Weight range (kg)	Feed (g/d)	Berberine Inclusion Rate mg/g	Daily Berberine Intake mg/d	Daily Berberine Intake mg/kg bw/day
Broiler, Male (a)	3.08 – 3.37	220.5	0.03	6.62	1.96 - 2.15
Broiler, Female (b)	2.56 – 2.78	200.3	0.03	6.01	2.16 - 2.35

(a) Rostagno et al, 2017, Table 2.20: Nutritional requirements of male broilers -Regular-average performance (pg. 292);
(b) Rostagno et al, 2017, Table 2.23: Nutritional requirements of female broilers - Regular-average performance (pg. 295) Rostagno et al, 2017

Estimated Human Exposure

Chicken Consumption

The EDI of chicken (muscles, tissues and organs) was based on food consumption records collected in the What We Eat in America (WWEIA) component of the NHANES conducted in 2013-2014 and 2015-2016 (WWEIA, NHANES 2013-2016). This continuous survey uses a complex multistage probability sample designed to be representative of the civilian U.S. population (NCHS, 2016, 2018). The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the U.S. Statistical weights are provided by the National Center for Health Statistics (NCHS) to adjust for the differential probabilities of selection.

As part of the examination, trained dietary interviewers collected detailed information on all foods and beverages consumed by respondents in the previous 24-hour time period (midnight to midnight). A second dietary recall was administered by telephone three to ten days after the first dietary interview, but not on the same day of the week as the first interview. The dietary component of the survey is conducted as a partnership between the USDA and the U.S. Department of Health and Human Services (DHHS). DHHS is responsible for the sample design and data collection, and USDA is responsible for the survey's dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. A total of 13,600 individuals' ages 2 years and older in the survey period 2013-2016 provided 2 complete days of dietary recalls.

Identification of Chicken Products in the Diet

Exponent used the Food and Nutrient Database for Dietary Studies (FNDDS) in combination with the Food Patterns Equivalents Ingredients Database (FPID), both developed by the USDA, to identify amounts of chicken-derived components in foods consumed by the U.S. population.

The FNDDS translates each food code reported consumed in WWEIA, NHANES into one or more food ingredients. For each food code reported in WWEIA, NHANES, USDA uses the proportion (by weight) of ingredients in combination with nutrient data for each ingredient to

develop nutrient concentrations for each food code. The ingredients used in FNDDS include primarily single component foods though some ingredients in FNDDS represent mixtures. Examples of chicken-derived single component ingredients used by USDA in FNDDS include chicken identified by part (e.g., breast, drumstick, thigh, etc.). Examples of FNDDS ingredients representing mixtures containing chicken-derived components include 'Fast foods, crispy chicken filet sandwich, with lettuce and mayonnaise,' 'Fast foods, chicken tenders,' and 'Fast foods, quesadilla, with chicken.' Exponent applied FNDDS 2015-2016 recipes (USDA, 2018b) to process dietary recall data reported in WWEIA, NHANES 2013-2016 and FNDDS 2013-2014 recipes (USDA, 2016) for foods that were only reported consumed in WWEIA, NHANES 2013-2014.

The FPID (which is a variation of the Food Patterns Equivalents Database (FPED) developed by USDA), further translates the FNDDS ingredients into equivalents of USDA food pattern groups. Food pattern groups corresponding to chicken-derived components include total protein foods with pertinent subgroups for poultry and organ meat. Exponent applied FPID 2015-2016 (USDA, 2018a) for FNDDS ingredients reported consumed during WWEIA, NHANES 2013-2016 and FPID 2013-2014 (USDA, 2017) for FNDDS ingredients reported consumed only during WWEIA, NHANES 2013-2014.

For FNDDS ingredients corresponding to a single-component food (i.e., chicken breast), 100% of the FNNDS ingredient weight was included in the analysis. Meat ingredients identified as a combination of beef, pork or poultry were assumed to contain equal proportions of each specified type. For FNDDS ingredients corresponding to a food mixture (e.g., chicken filet sandwich) rather than a single-component food, the weight of chicken per 100 grams of FNDDS ingredient mixture was calculated by taking the ratio of the poultry food pattern component concentration in the ingredient mixture (e.g., chicken filet sandwich) relative to the concentration of the poultry food pattern component in an ingredient corresponding to the single-component food (e.g., chicken breast). The proportion of chicken (by weight) was calculated for each FNDDS ingredient and WWEIA/NHANES food code and only the portion corresponding to chicken within the food was included in the assessment. The FNDDS ingredients with chicken-derived components used in the estimates of intake are provided in **Appendix H**.

The analysis was limited to individuals who provided two complete and reliable dietary recalls as determined by NCHS. The 2-day average intakes by each individual were estimated using Exponent's Foods Analysis and Residue Evaluation (FARE® version 13.05) software. Exponent used the statistically weighted values in the analysis. The statistical weights compensate for variable probabilities of selection, adjust for non-response, and provide intake estimates that are representative of the U.S. population. Per user estimated intakes, on a bodyweight basis, using each individual's measured body weight, for children aged 2-5 years and the total U.S. population aged 2+ years are summarized in **Table 7**.

Table 7. Two-Day Average Estimated Daily Intake of Chicken; NHANES 2013-2016

	Unwtd N[a]	% User	Per User (g/day)		Per User (g/kg-bw/day)	
			Mean	90th Percentile	Mean	90th Percentile
Children 2-5 y	796	71	35.4	74.0	2.12	4.55
U.S. 2+ y	9,468	68	59.3	127.2	0.92	1.94

[a] Unweighted number of users; % user and per user estimates derived using the statistical weights provided by the NCHS.

Berberine Residue in Broiler Chickens and Estimated Daily Intake

As summarized under the safety data section of this dossier, a residue study is available for broiler chickens fed IRP Health's berberine in the diet at concentrations of 0, 0.03 ("low") or 0.3 g/kg ("high") (Yu et al., 2017). Results from the study showed that at the "low" berberine diet concentration of 0.03 g/kg, berberine residue levels of tissue in all cases were less than 2 ng/g (the limit of detection). At this lower concentration in feed, berberine residue levels in the liver were also below the limit of detection before and after washout.

The intended use level of berberine as a preservative in broiler chicken feed is 0.03 g/kg (30 mg/kg) of feed. Thus, for the purpose of the current human dietary exposure and safety assessment, the maximum residue level in broiler chicken tissues is assumed to be the limit of detection (2 ng/g). This maximum residue level is combined with the chicken intake estimates to develop the EDI of berberine as summarized below in **Table 8**.

Table 8. Two-day Average Estimated Daily Intake of Berberine; NHANES 2013-2016

	Unwtd N[a]	% User	Per User (mg/day)		Per User (mg/kg-bw/day)	
			Mean	90th Percentile	Mean	90th Percentile
Children 2-5 y	796	71	7.1E-05	1.5E-04	4.2E-06	9.1E-06
U.S. 2+ y	9,468	68	1.2E-04	2.5E-04	1.8E-06	3.9E-06

[a] Unweighted number of users; % user and per user estimates derived using the statistical weights provided by the NCHS.

Part 4. Self-Limiting Levels of Use (21 CFR § 570.240)

Berberine is intended to be used as a preservative in the feed of broiler chickens. The maximum use level is 0.03 g/kg (30 mg/kg) to preserve feed from yeast and mold. Self-limiting levels of use are not applicable to this notice.

Part 5. Experience Based on Common Use in Food before 1958 (21 CFR § 570.245)

IRP Health's conclusion of GRAS status for the intended use of berberine as a preservative in feed for broiler chickens is based on scientific procedures in accordance with 21 CFR 570.30(a) and (b)

Experience based on common use in food before 1958 is not applicable to this notice.

Part 6. Narrative (21 CFR § 570.250)

Introduction

Berberine (CAS 633-65-8, anhydrous) (5,6-Dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-α] quinolizinium chloride hydrate), a benzylisoquinoline alkaloid, is an active constituent in numerous medicinal plants including *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (Coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), and an Indian species *Berberis aristata* (Tree turmeric). *B. aristata*, (fam. Berberidaceae) (Kumar et al., 2015). It has been used in Ayurvedic and Chinese medicine for its antimicrobial, antiprotozoal, antidiarrheal and antitrachoma activity (Kumar et al., 2015). IRP Health's berberine is extracted from dry bark of *Phellodendron chinense.* This plant has been widely used in traditional Chinese medicine over hundreds of years.

Literature searches were conducted in December 2020 using PubMed, with supplemental searches performed in GoogleScholar to identify studies containing information pertinent to the safety of berberine. Additional databases that were searched included the National Toxicology Program (NTP), EFSA, FDA, Food Standards Australia New Zealand (FSANZ), and the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA). The following search strings were used in PubMed and returned the following results:

Table 9. PubMed Search Strings That Were Used For Safety Data Literature Search

Search String	**Search Filter(s)**	**# Citations Returned**
berberine [ti] AND (safe* OR toxicity OR toxicol* OR acute OR chronic OR subchronic OR mutagenicity OR genotox* OR dart [subset])	English, Title	627
berberine [ti] AND ("randomized controlled trial" [pt] OR "controlled clinical trial" [PT] OR "Clinical Trial"[pt] OR randomized OR randomised OR "controlled trial" OR "clinical trial" OR crossover OR cross-over OR pilot)	English, Title	160
berberine [ti] AND (review OR meta-analysis OR meta-analysis)	English, Title	150
berberine [ti] AND poultry	English, Title	8

Citation titles were returned and reviewed, followed by a review of abstracts in cases where the title did not provide sufficient information to judge the relevance of a publication. Inclusion criteria for papers that were selected for further evaluation included those that contained relevant safety data and efficacy studies that reported safety related outcomes. Studies that were excluded from further review were papers that utilized *in situ* or *in vitro* systems (except for *in vitro* genotoxicity studies), evaluated methodologies, performed chemical analyses, examined synergistic effects, applied a non-relevant route of exposure, or were efficacy studies that did not report or explore any toxicity-related parameters.

Safety Data on Target Species – Chicken

Pharmacokinetics Studies

Haipeng et al. (2009) evaluated the pharmacokinetics of berberine hydrochloride (97.58% content, (b) (4) administered to laying hens (n=16) that were randomized to receive a single oral or intravenous dose of 50 or 3 mg/kg, respectively, of berberine hydrochloride. After the test material was administered, blood samples were collected from the wing vein at multiple time points over a 12-hour period. Plasma concentrations of berberine were measured by high-performance liquid chromatography (HPLC).

Overall results revealed that orally administered berberine was poorly absorbed in chickens, as indicated by the low absolute bioavailability of 2.03% and Cmax of 4.09 µg/l. As expected, intravenously administered berberine resulted in rapid absorption, distribution, and elimination of berberine. The time-concentration curves for oral and intravenous routes of exposure to berberine conformed to the two- and three-compartment open models, respectively. Data for each of the measured pharmacokinetic parameters are described in **Table 10**.

Table 10. Pharmacokinetic Parameters of a Single Dose of Intravenously and Orally Administered Berberine Hydrochloride (X±S)

Parameter[2]	Unit	Intravenous Injection	Oral Administration
A	μg/l	158.37 ± 101.88	25.76 ± 30.83
A	h^{-1}	27.17 ± 25.77	0.43 ± 0.18
B	μg/l	29.87 ± 22.00	19.16 ± 28.59
B	h^{-1}	2.09 ± 0.96	0.22 ± 0.10
G	μg/l	8.68 ± 3.18	---
Γ	h^{-1}	0.20 ± 0.06	---
K_a	h^{-1}	---	1.14 ± 0.10
t 1/2α	H	0.09 ± 0.12	1.87 ± 0.76
t 1/2β	H	0.41 ± 0.24	4.18 ± 3.14
t 1/2γ	H	3.66 ± 1.06	---
t 1/2ka	H	---	0.89 ± 0.46
V_c	l/kg	25.49 ± 21.77	---
CL	l/h/kg	43.20 ± 16.21	---
Cmax	μg/l	---	4.09 ± 0.11
Tmax	H	---	2.64 ± 0.63
AUC	μg/l/h	78.92 ± 30.58	26.18 ± 10.73
F	%	---	2.03

Tissue Residues Study

A residue study was conducted in n=180 (divided into 18 pens, 10 birds/pen) broiler chickens fed with berberine (IRP001 by (b) (4) in the diet at concentrations of 0, 0.03 ("low") or 0.3 ("high") g/kg (Yu et al., 2017). Broilers were given diets containing berberine for either 28 or 35 days. The group receiving berberine for 35 days was either sacrificed at the end of the period or fed untreated feed for 7 days. The group treated for 28 days was fed an untreated diet for 14 days. At the end of the study and following euthanasia, samples were collected from three regions of muscle tissue (breast, upper and lower thigh).

Residues were 5.5 and 6.1 ng/g for thigh and breast tissue without a washout period for animals receiving the higher dietary concentration (0.3 g/kg) for 35 days. Liver residues averaged 6.5 ng/g after the 7 day wash out at the high concentration but were below the validated limit of quantitation of 5 ng/g after 14 days of wash out for all tissues.

[2] Exact parameters stated as reported in Haipeng et al. (2009), certified translation from Chinese to English; definitions for abbreviations were not translated

Results from the study showed that at the low berberine diet concentration of 0.03g/kg berberine residue levels of tissue in all cases were less than 2 ng/g (the limit of detection). At this lower concentration in feed, berberine residue levels in the liver were also below the limit of detection before and after washout.

Feeding Studies

Yang et al. (2019) investigated the effects of orally administered berberine (purity >98% (b) (4) (b) (4) on growth performance, immunity, and antioxidative capacity in male broilers challenged with lipopolysaccharide (LPS). Male broilers (n=120; one-day-old in age) were randomized into two treatment groups that were each divided into two subgroups of six replicate pens consisting of 5 birds/replicate. A 2x2 factorial experimental design was employed based on treatments with 0 (basal diet) or 60 mg/kg diet of berberine and a saline (9 g/l w/v) or LPS (1.5 mg/kg bw) challenge that was administered intraperitoneally on 14, 16, 18, and 20 days of age. All birds were euthanized on day 21. Measured parameters included growth performance, antioxidant activity, immunological tests, hematological and serum lipid analyses. Only berberine-specific safety data are summarized herein. Overall results revealed that 60 mg/kg diet of berberine fed to male broilers did not cause any significant differences in any of the measured parameters compared to the basal diet.

Xiao et al. (2018) investigated the safety of berberine chloride sourced from (b) (4) (b) (4) as a feed ingredient in broiler chickens. Male boiler Cobb chickens were divided into pens of 20 individuals. Three treatment groups and one control group were each replicated in 8 blocks. Treatment groups consisted of the following:

- No Additive (control)
- Berberine: 0.3 g/kg
- Berberine: 0.1 g/kg
- Berberine: 0.03 g/kg

Feed was provided *ad libitum* and quantities used to prepare treatment batches were documented. The temperature was optimized to the age of the animals and illumination was provided by fluorescent bulbs placed above the pens with 21 hours of light per day from day 0 to 42. Animals and housing facilities were inspected twice daily, observing and recording general health status, feed and water supply, and temperature. Birds found dead during the study were

noted on the Daily Mortality Record and were not replaced. Bird weights by pen were recorded on days 0, 21, 35, and 42. Non-consumed feed was weighed by pen on days 21, 35, and 42. On day 42, two birds from each pen were euthanized and liver lobes were collected for analysis. There were no dose-dependent differences between treatment groups and control birds in terms of feed intake, food consumption, or weight gain. On day 21, birds receiving the two lower doses of berberine had significantly higher feed uptake compared to the control. However, this effect was not seen at the highest dose. Similarly, weight gain was significantly higher in the birds receiving the middle dose of berberine, but not the low or high dose. There was no difference between berberine and control birds on day 35 or 42 with respect to feed intake, feed consumption ratio (FCR) or average weight gain. The overall percent-mortality was lower in the birds receiving the low and high dose of berberine (0.5% and 0.8%, respectively) compared to control birds (2.8% mortality).

Results of the histological analysis appear to indicate that the test substance had no significant histological effects, although statistical analyses were not carried out. Liver effects were scored by individual in terms of their severity and the mean score for each treatment was calculated; the percentage of birds with each effect was also recorded. The average severity of portal cholangitis was higher in the middle and high dose berberine groups (mean score of 2.2 and 2.0 in the mid and high dose groups, respectively) compared to controls (mean 1.4). Mineralization was also more severe at the higher two doses of berberine (mean scores of 0.3 and 0.4 in the mid and high dose groups, respectively) compared to control (mean 0.2). However, the cumulative pathology scores for the low, mid and high dose groups were not dose-dependent (11.2, 10.6, 9.8, respectively), which further indicates that berberine's effect on the liver was not significant. Overall, there are no significant differences between controls and treatments on food intake, FCR, and weight gain. No differences were observed in the liver lesion index in controls and berberine-treated groups. Lesions such as mild lymphocytic hepatitis in the portal regions and extramedullary hematopoiesis that were reported in the liver histology studies were within normal limits for broiler chickens in a production environment based on similar percent occurrences of lesions in non-treated controls and berberine-treated groups. Therefore, the study authors concluded that there is no safety concern for chicken broilers at the study test doses.

In a separate unpublished study (Mathis, 2017), the safety of berberine was evaluated in n=10 male Cobb chickens/group that were fed 0, 0.05, 0.5 or 1.0 g/kg of berberine for 42 days. Examinations included food and water availability as well as health and environmental conditions (i.e., temperature). At the end of the study and following euthanasia, tissue samples from n=5 chickens/group were collected from the upper, mid- and lower gut sections. Overall results revealed that chicken appearance, feed intake, weight gain and mortality were normal at all tested doses. Liver and lower gastrointestinal histology also appeared to be normal for the 5 chickens per group that were analyzed. Furthermore, no treatment-related adverse effects were observed in chickens receiving diets of up the highest tested dose of 1.0 g/kg berberine.

Summary of safety data for chickens: Orally administered berberine was poorly absorbed in chickens, as indicated by the low absolute bioavailability of 2.03% and Cmax of 4.09 µg/l. As expected, intravenously administered berberine resulted in rapid absorption, distribution, and elimination of berberine. Berberine was not detected (LOD of 2 ng/g) in tissues of chickens fed a low level of berberine in their diet (0.03g berberine/kg feed). Feeding studies showed no safety concern in chickens fed with berberine containing feed at concentration up to 1 g/kg. IRP Health's proposed maximum use level of berberine in chicken feed is 0.03g/kg and is, therefore, safe for broiler chickens.

Safety Data Relevant for Human

Absorption, Distribution, Metabolism, and Excretion

Absorption

The pharmacokinetic profile of berberine and its metabolites has been well-studied in humans and rats. It has low oral bioavailability which is attributed to its poor aqueous solubility. Fasted Sprague-Dawley rats dosed with berberine by the intravenous, intragastric, intraductal and intraduodenal routes showed that the gastrointestinal bioavailability was 0.50 to 0.51% (Liu et al., 2010). Major metabolites included the products of deethylation, demethylenation and hydrolysis. Sulfate and glucuronide metabolites of first pass metabolism were also observed in rats. Extensive intestinal and hepatic metabolism limits both the area under the curve (AUC) and C_{max} after oral dosing. The time to C_{max} (C_{max} = 1.1 ng/ml) was 3.0 hours and the AUC was 0.15

ng.h/ml.kg after dosing of healthy subjects with 132 mg of berberine (Gupta et al., 2010). Oral dosing of male Sprague-Dawley rats with 200 mg/kg bw by gavage showed that berberine concentrations in liver, plasma, kidneys, fat and muscle peaked within 12 hours (Tan et al., 2013). Metabolite concentrations also peaked within 24 hours after dosing in all tissues. Berberine and metabolite concentrations declined most rapidly in the liver, the site of the highest initial concentrations. The authors concluded that the relative stability of berberine and metabolites in tissues explains reported clinical activity *in vivo* despite low plasma concentrations.

Distribution

Although berberine is present at a very low level in blood and its bioavailability was reported to be less than 1%, the pharmacological effect of berberine can be correlated with its high tissue distribution. The organ distribution of berberine is rapid with maximum distribution in the liver, followed by the kidneys, muscle, lungs, brain, heart, and pancreas, with the lowest level of distribution occurring in fat (as reviewed in Kumar et al., 2015).

Metabolism

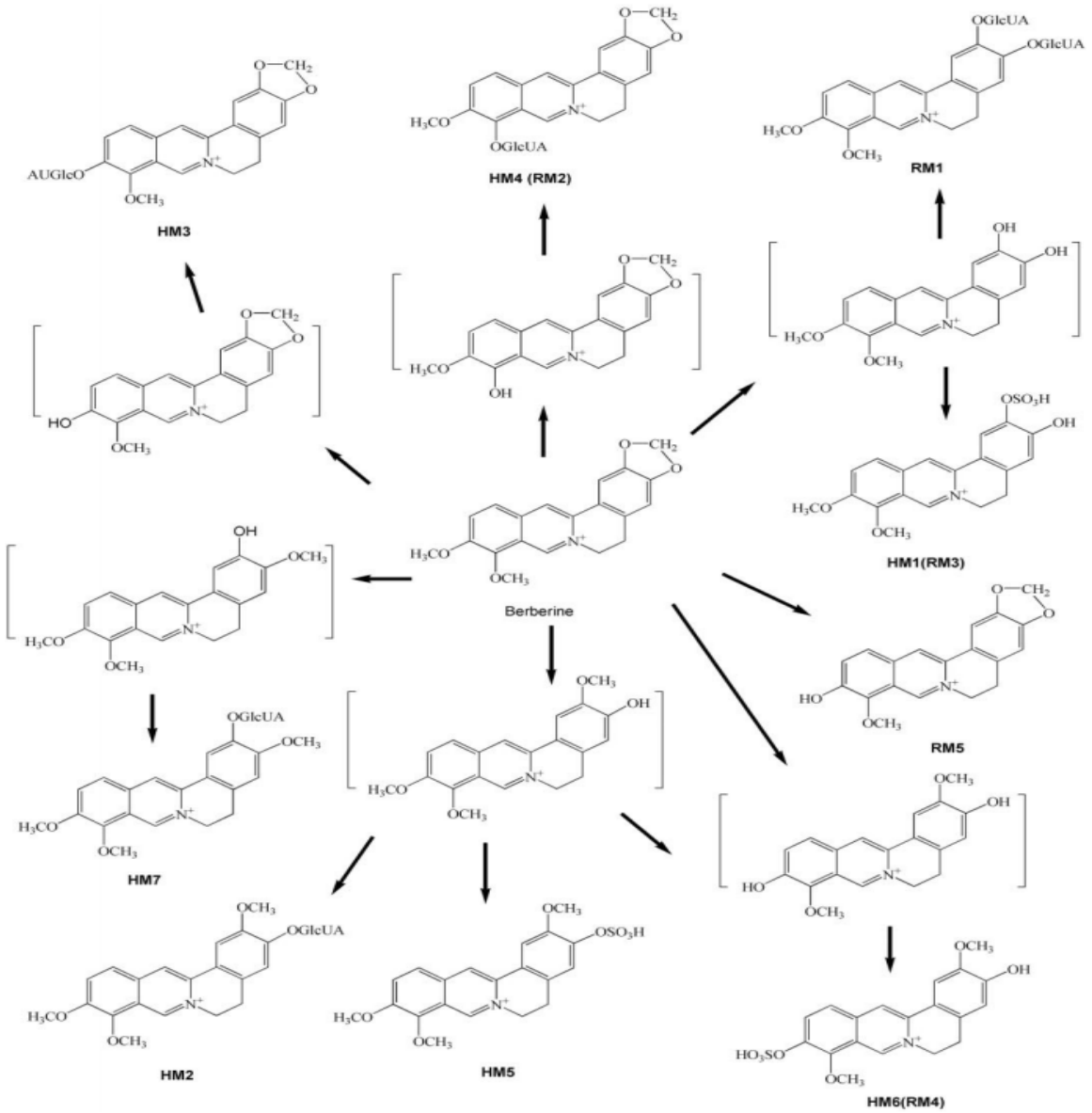
Berberine is metabolized in the liver, in both rats and humans, undergoing demethylation in Phase I metabolism, followed by conjugation with glucuronic acid or sulfuric acid to form Phase II metabolites (Kumar et al., 2015). A study of the metabolism of berberine chloride was conducted in five heathy male human volunteers (Pan et al., 2002). Volunteers received 100 mg tablets for three days. Urine samples were collected and analyzed. Three metabolites were identified in the urine: jatrorhizine-3-sulfate, demethylene berberine 2-sulfate and thalifendine-10-sulfate. Comparison of urinary metabolites of berberine in humans and Wistar rats showed that berberine undergoes similar biotransformation in humans and rats (Qiu et al., 2008). The metabolites of berberine are shown in Figure 4 below.

Excretion

Ma et al. (2013) investigated the excretion of berberine and its metabolites in rats following an orally administered dose of 200 mg/kg berberine. The total recovery rate of berberine was 22.83% (19.07% of berberine prototype and 3.76% of berberine metabolites). Recovery in the bile (24 hr),

urine (48 hr), and feces (48 hr) was 9.2×10^{-6}, 0.0939, and 22.74%, respectively. Berberine (83%) was excreted in the form of thalifendine from bile; thalifendine and berberrubine were the major metabolites (78%) excreted in urine. Berberine (84%) and its metabolites were primarily found in the feces.

Figure 4. Berberine metabolic pathway in rats and humans (Qiu et al., 2008)



Pre-Clinical Safety Data

Acute Toxicity

Acute toxicity data have been reported for berberine and berberine sulfate from oral and intraperitoneal (i.p.) routes of exposure in mice and rats. Oral and i.p. LD50 values of 329 and 23 mg/kg bw, respectively, have been reported for berberine exposure in mice (Kulkarni et al., 1972 as cited by NTP, 2010 and Singh and Sharma, 2018). Yi et al. (2012) reported an oral LD50 of 713.57 mg/kg bw in mice. Berberine sulfate LD50 values that have been reported in rats for oral and i.p. exposure routes are >1000 and 88.5 mg/kg bw, respectively (Kowalewski et al., 1975, as cited by NTP, 2010). Kulkarni et al. (1972, as cited by NTP, 2010 and Singh and Sharma, 2018) reported an i.p. LD50 of 88.5 mg/kg bw in albino rats.

Short-term Toxicity

Berberine orally administered to cats for 8-10 days caused vomiting and lethality at a dose of 100 mg/kg bw/day, while 50-100 mg/kg bw of berberine sulfate resulted in hemorrhagic inflammatory issues in the small and large intestine (Singh and Sharma, 2018).

Sub-chronic Toxicity

In accordance with the Organisation for Economic Co-operation and Development (OECD) 408 and the China Food and Drug Administration (CFDA) testing guidelines, Ning et al. (2015) evaluated the subchronic toxicity of the fibrous root of *Rhizoma Coptidis* (FRC) containing 1.20% berberine n=10 Sprague-Dawley rats/sex/group that were administered oral doses of 0.3% CMC (control; 10 ml/kg bw) or FRC at doses of 940, 1,880, and 3,760 mg/kg bw/day for 90 days. Standard toxicological parameters were measured. Overall results revealed histological liver (hepatocyte degeneration) and lung (inflammatory cell infiltrates) lesions as well as significant increases in serum levels of alanine-aminotransferase and aspartate-aminotransferase liver enzymes at the highest tested dose. Thus, the study authors determined the NOAEL of FRC to be 1,880 mg/kg bw/day (equivalent to 22.56 mg/kg bw/day of berberine) based on the effects observed at the highest tested dose.

Yi et al. (2012) conducted a 90-day non-OECD guideline study in n=5 Sprague-Dawley rats/sex that were administered 156 mg/kg bw/day (single tested dose) of berberine in the diet. Compared to controls, no toxicologically significant findings were observed from berberine exposure for multiple measured parameters such as mortality, clinical signs, body weight changes, organ weights, urinalysis, histopathogy, hematology, and gross necropsy.

NTP (2010) Sub-chronic Toxicity Studies

Repeat dose studies of berberine are available that assess berberine primarily as a component of goldenseal root powder (NTP, 2010). Berberine is present in the root powder at concentrations ranging from 1.5 to 4.0%. Other isoquinoline alkaloids are also present in the root powder at significant concentrations. Hydrastine is found at a similar concentration range and canadine and sideroxylin are present at lower levels. These alkaloids would be expected to contribute to the toxicity of goldenseal.

Ninety-day studies of goldenseal were conducted in F344/N rats and B6C3F$_1$ mice prior to the carcinogenicity studies described below to facilitate dose-setting (NTP, 2010). Ten male and ten female rats and mice were given diets containing 0, 3,121, 6,250, 12,500, 25,000 or 50,000 ppm of goldenseal root powder (3.45% berberine). Additional groups of 10 rats/sex/group were treated for 23 days for clinical pathology examinations. In rats, the equivalent daily doses were approximately 0, 255, 500, 1,000, 2,020, or 4,060 mg/kg bw/day in males and 0, 260, 500, 1,030, 2,070, or 4,100 mg/kg bw/day in females. Equivalent daily doses in mice were approximately 0, 680, 1,360, 2,260, 5,370, or 10,550 in males and 0, 590, 1,250, 2,345, 4,790, or 10,740 mg/kg bw/day in females (NTP, 2010). Examinations included clinical signs, body weight, food consumption, organ weights, hematological analyses, clinical chemistry, histopathology, macroscopic observations, sperm motility, and estrous cycle length.

All rats survived throughout the study, and no differences in body weight or food consumption were observed between controls and treated animals. No significant differences were observed in reproductive organ weights, sperm parameters, or estrous cyclicity between controls and treated animals. In rats, slight and transient increases in the erythron were seen in males and females at the two highest dose levels. Small but progressive increases were observed in both

sexes for total protein and serum albumin in rats at 12,500 ppm and 25,000 ppm doses, respectively. Absolute and relative liver weights were significantly increased at concentrations ≥ 6,250 ppm in males and at all tested doses in females. According to the study authors, the incidences of hepatocyte centrilobular hypertrophy and increased liver weights at 25,000 and 50,000 ppm doses were not severe enough to adversely affect a 2-year study. Thus, the exposure concentrations that were selected for the 2-year study were 3,000, 9,000, and 25,000 ppm. Based on the increased absolute and relative liver weights that were observed in females at all tested doses in this 90-day rat study, a lowest observed adverse effect level of 3,121 ppm of goldenseal (108 ppm of berberine) could be inferred. This is equivalent to ~ 9 mg/kg bw/day of berberine.

All mice survived throughout the study. No changes in food consumption, hematology, reproductive parameters, or treatment-related clinical findings were observed compared to controls. Final body weights were significantly less than controls at the highest dose in males and at ≥ 25,000 ppm doses in females. Absolute and relative liver weights were significantly increased in males at ≥ 12,500 ppm doses and at 25,000 and 50,000 ppm doses in females. These findings were consistent with increased incidences of hepatocyte hypertrophy at the stated doses. Absolute epididymal weights were increased in males at the highest tested dose compared to controls. Treatment-related glycogen depletion was seen histologically in female mice at concentrations ≥ 12,500 ppm and at the 50,000 ppm dose in males. Based on the hepatocyte centrilobular hypertrophy and decreased final body weights that were observed at the highest tested dose, the concentrations that were selected for the 2-year study were 3,000, 9,000, and 25,000 ppm. Based on the liver weight findings in this 90-day mouse study, a NOAEL of 6,250 ppm of goldenseal (216 ppm of berberine) could be inferred. This is equivalent to ~ 47 (males) and 43 (females) mg/kg bw/day of berberine.

Carcinogenicity and Long-term Toxicity

NTP – Mice Study

Goldenseal root powder containing berberine and several other alkaloids including canadine and hydrastine was assayed for carcinogenicity in F344/N rats and $B6C3F_1$ mice (NTP, 2010). Fifty male and female mice were fed diets containing goldenseal at concentrations of 3,000, 9,000 and 25,000 ppm equivalent to 0, 375, 1,120 or 3,275 mg/kg bw/day for males and 0, 330, 1,000 or

2,875 mg/kg bw/day for females for two years. The berberine content of the goldenseal root powder was 3.89%, resulting in equivalent daily dose of 0, 15, 44, and 145 mg/kg bw/day and 13, 39, and 112 mg/kg bw/day of berberine in males and females, respectively. Examinations included body weights, food consumption, clinical signs, and histopathology.

In male mice, a positive trend was observed for neoplastic effects that included hepatoblastoma (1, 2, 1, and 6 observed in 50 animals per group for 0, 3,000, 9,000, and 25,000 ppm doses, respectively) and dose-dependent increases in multiple hepatocellular adenomas (3, 5, 11, and 18 observed in 50 animals per group for 0, 3,000, 9,000, and 25,000 ppm doses, respectively). Pairwise comparisons between controls and treated animals in the incidence of hepatoblastomas did not reveal any statistically significant differences. A summary of the liver tumor incidence in male mice is described in **Table 11** below. The incidence of non-neoplastic hepatocellular eosinophilic foci was significantly increased at the highest tested dose in male mice. The survival of the female mice at the mid dose was lower than that observed in female controls; however, no dose-dependent trends in survival were observed. Moreover, no effect on survival was seen at the low dose of goldenseal in females (equivalent dose level of berberine of 13 mg/kg bw/day). Overall, the NTP review (2010) concluded that there was "*some evidence of carcinogenic activity* of goldenseal root powder in male B6CF31 mice based on the increased incidences of hepatoblastoma and multiple hepatocellular adenoma." Conversely, there was no evidence of carcinogenic activity in females at all tested doses.

Table 11. Liver Tumor Incidence (Males) in Goldenseal Mouse Carcinogenicity Study (Equivalent Dose Level of Berberine in Goldenseal)

Males				
Equivalent Dose level (mg of berberine/kg bw/day)	0	14.59	43.57	127.4
Hepatocellular adenoma	22	16	23	29
Hepatocellular carcinoma	8	14	15	12

NTP Rat Study

In a two year study conducted in rats, 50 male and 50 female rats per group were fed diets containing goldenseal root powder concentrations of 0, 3,000, 9,000, or 25,000 ppm (0, 135, 400 or 1,175 mg/kg bw/day for males and 0, 150, 470 or 1,340 mg/kg bw/day for females). Based on a berberine content of 3.89%, equivalent dose of berberine are approximately 5, 16, and 46 mg/kg/day and 6, 18, and 52 mg/kg bw/day in males and females, respectively. Examinations included body weights, food consumption, clinical signs, and histopathology.

No toxicologically significant findings were observed between controls and treated groups in survival, clinical signs and food consumption. Decreased body weights were observed in females that were administered the test material at 9,000 and 25,000 ppm doses. The tumor incidences of benign hepatocellular tumors and hepatocellular adenomas were increased at the high dose level in males and females (see **Table 12**, below). The incidence of eosinophilic foci in hepatocytes was also increased in treated animals in both sexes.

Table 12. Liver Tumor Incidence in Goldenseal Rat Carcinogenicity Study (Equivalent Dose of Berberine in Goldenseal)

Males				
Equivalent Dose level (mg of berberine/kg bw/day)	0	5.25	15.75	45.7
Hepatocellular adenoma	1*	1	2	10**
Hepatocellular carcinoma	0	0	0	1
Females				
Equivalent Dose level (mg of berberine/kg bw/day)	0	5.8	18.28	52.1
Hepatocellular adenoma	0	0	1	8**

*N = 50 for all dose levels
** P=0.003 and P=0.004 for males and females, respectively; poly-3 test

The high dose rat incidence of hepatocellular carcinomas in males (1/50 or 2%) was unusual compared with the historical control incidence (1/1250 or 0.08%) but not significantly increased compared with the concurrent control group. The historical incidence of hepatocellular adenoma for 2-year feeding studies in controls ranged from 0-6% based on historical control data. The incidence of hepatocellular adenoma in high-dose treated males (20%) from this study was outside of the historical control incidence in NTP studies with this strain of rat. The

incidence of hepatocellular adenomas and carcinomas combined in males was significant ($p<0.001$) in males and outside of the historical range of 0-6% but this was primarily due to the increase in adenomas at the high dose level. The historical incidence of heptacellular adenoma for feeding studies was reported to range from 0-4% in females; the incidence of hepatocellular adenoma in high-dose treated females (16%) from this study exceeded historical control data. Thus, based on the results, goldenseal was associated with an increased incidence of benign hepatocellular tumors at the two highest dose levels in the rat carcinogenicity study.

Although berberine may be associated with an increase in benign liver tumors in the rat when tested as a component of the goldenseal root powder, these effects may be secondary to changes in liver function or due to other components of the goldenseal mixture. The absence of berberine induced genotoxicity or developmental toxicity (as described in the following sections) suggests that berberine is not a direct acting carcinogen[3].

There are no other long-term studies of berberine. Evidence for the carcinogenicity for berberine is limited to studies in which berberine is a minor component of goldenseal and the weak potential of berberine to inhibit topoisomerase (see *Genotoxicity*, below).

Developmental and Reproductive Toxicity

Sprague-Dawley rats received diets containing 0, 3,125, 6,250, 12,500 or 18,400 ppm of goldenseal root powder on gestation days 6 to 20 (NTP, 2003). The test material contained 5% berberine, resulting in equivalent berberine doses of 0, 10, 21, 42, and 61 mg/kg bw/day. Twenty-five timed pregnant animals were used per dose level. Maternal liver weights were increased at exposures of 6,250 ppm and greater. Relative food consumption and body weight gain were transiently reduced in dams during the early treatment period, which the study authors attributed to palatability issues; increased water consumption was reported at the end of gestations. No adverse treatment-related effects were observed in multiple fetal endpoints, including prenatal mortality, live litter size, fetal body weight, percent male fetuses/litter,

[3] The etiologies of developmental toxicity have many common pathways with carcinogenicity. For example, epigenetic changes induced *in utero* may increase susceptibility to cancer in aging animals (Ho et al., 2017; Cheong et al. 2017). Cytotoxicity and alterations in rates of cell division may be early events in both carcinogenicity and developmental toxicity.

malformations or variations. Based on the results, the study authors reported a developmental toxicity NOAEL of ≥ 18,400 ppm for goldenseal that is equivalent to ≥ 61 mg/kg bw/day of berberine. The study authors did not report a NOAEL for maternal toxicity.

Time pregnant Swiss-Webster mice (25/group) received diets containing either 0, 3,125, 12,500 or 50,000 ppm of goldenseal root powder on days 6 to 17 of gestation (NTP, 2002). This corresponded to ingested berberine doses of 0, 26, 102 and 387 mg/kg bw/day. Absolute liver weight was increased at the high dose level but occurred in the absence of treatment-related histopathological lesions. Water consumption was also increased at the highest dose level. Dose-related staining of the fur was observed at all dose levels and increased maternal weight gain was reported at the low and mid doses. The study authors reported that overall, during the treatment period, maternal body weight gain, gestational body weight gain, gravid uterine weight, and corrected weight gain were unaffected. Fetal examinations did not reveal any significant treatment-related effects on mortality, litter size, percent male fetuses/litter, malformations, or variations. The average fetal body weight/litter was significantly reduced at the high dose level. There was no other indication of developmental toxicity. The study authors determined the NOAEL for developmental toxicity to be 12,500 ppm, equivalent to 102 mg/kg bw/day of berberine.

In the study by Jahnke et al., (2006), which was sponsored by the National Institute of Environmental Health Sciences (NIEHS)-NTP, berberine chloride dihydrate (BCD) was evaluated for developmental toxicity in rats and mice. BCD was administered in the feed to 25 timed-mated female Sprague–Dawley (CD) rats per dose group (0, 3,625, 7,250, or 14,500 ppm; on gestational days [GD] 6–20), and 25 female Swiss Albino (CD-1) mice (0, 3,500, 5,250, or 7,000 ppm; on GD 6–17). Ingested doses were reported in the study as 0, 282, 531, and 1,313 mg/kg bw/day (rats) and 0, 569, 841, and 1,155 mg/kg bw/day (mice). There were no maternal deaths, and no adverse clinical signs were observed in maternal rats or mice. The rat maternal LOAEL, based on reduced maternal weight gain of ~9%, which was statistically significant, was 7,250 ppm. The rat maternal NOAEL was therefore 3,625 ppm, or 282 mg/kg bw/day of BCD, equivalent to 223 mg/kg bw/day of berberine chloride. The rat developmental toxicity LOAEL, based on reduced fetal body weight per litter, was 14,500 ppm, and accordingly, the rat

developmental NOAEL for the dietary developmental study was 7,250 ppm, or 531 mg/kg bw/day of BCD, equivalent to 429 mg/kg bw/day of berberine chloride. In the mouse study, maternal and developmental toxicity LOAELs were either 5,250 ppm, or 841 mg/kg bw/day for increased water consumption in dams, and for higher incidence of cleft palate in the high dose group litters. However, the study authors concluded that this LOAEL for developmental toxicity was equivocal. The basis of this equivocal designation was the observation that the increased incidence was clear at the highest dose, and demonstrated a pattern of dose-response between the low dose and the intermediate dose group, but that the increased incidence in the intermediate dose group was slight, and complicated by a high known rate of background cleft palate in this strain of mice.

Due to scattering of feed in the high dose groups of the developmental study by Jahnke et al. (2006), a gavage study with a single dose at 1,000 mg/kg bw/day and a control group was conducted in both species and reported in the same publication. In maternal but not fetal rats, adverse effects were noted. The maternal toxicity LOAEL in rats was the same as that reported for the dietary study, 7,250 ppm (531 mg/kg bw/day), based on decreased body weight and gains in the maternal animals. The developmental toxicity NOAEL from the follow-up gavage study in rats was raised to 1,000 mg/kg bw/day BCD, equivalent to 792 mg/kg bw/day of berberine chloride, based on a lack of effects in the offspring.

In the follow-up gavage study in mice reported by Jahnke et al. (2006), 7 out of 25 of the gavage-treated female mice from the 1,000 mg/kg bw/day dose group died or were found moribund, not including mice dead or removed due to technician error in dosing. Surviving animals had increased relative water intake, and average fetal body weight per litter decreased 5–6% with no change in live litter size. Because effects were noted at the 1,000 mg/kg bw/day dose level in the follow-up gavage study, the maternal toxicity LOAEL for mice remained as 5,250 ppm (841 mg/kg/day) BCD, based on increased water consumption in dams in the dietary study. The developmental toxicity LOAEL for BCD in the mouse gavage study was 1,000 mg/kg/day BCD based on decreased fetal body weight, which is higher than the equivocal LOAEL from the dietary study in mice based on apparent dose response of cleft palate in the presence of high known background incidence. The overall developmental LOAEL in mice is

therefore 1,000 mg/kg bw/day of BCD, equivalent to 792 mg/kg bw/day of berberine chloride. Considering that in all studies conducted in the publication by Jahnke et al., fetal toxicity did not occur in the absence of maternal toxicity, berberine does not appear to be a specific developmental toxicant (Jahnke et al 2006).

Genotoxicity

In vitro bacterial system assays of goldenseal powder in *E. coli* and *S. typhimurium* were negative with and without metabolic activation. Berberine chloride was tested in an Ames study with and without metabolic activation and was found to be negative (NTP, 2010). The frequency of micronuclei was not increased in blood taken from male or female $B6C3F_1$ mice exposed to diets containing up to 50,000 ppm of goldenseal powder (approximately 400 mg/kg/day of berberine) (NTP, 2010). Berberine chloride was negative for micronucleus formation in $B6C3F_1$ mice dosed intraperitoneally three times at 24-hour intervals with a dose of 658 mg/kg bw (NTP, 2010). Similar findings were reported by Ning et al. (2015), whereby ICR mice administered oral doses of fibrous root of *Rhizoma Coptidis* (containing 1.20% berberine) up to 7000 mg/kg bw/day yielded negative results for micronucleus formation.

Berberine is known to inhibit topoisomerase I and II as well as DNA repair enzymes (as reviewed in NTP, 2010)). Berberine also appears to bind selectively to Adenine Thymine (AT)-rich sequences (Mazzini et al., 2003). Chen et al., (2013) concluded that the DNA damage is mediated with topoisomerase II, but not topoisomerase I. Berberine has little potential to directly damage DNA. The authors noted that DNA damage via topoisomerase inhibition occurs at a concentration of 3.125 micromolar which is almost 3-fold higher than the plasma concentration of 1.2 micromolar which is reached after a therapeutic oral dose of 300 mg (5 mg/kg bw/day for a 60 kg individual).

Antiproliferative properties of berberine were reported in Constitutive Androstane Receptor (CAR) over-expressed *in vitro* Hep G2 cells (Zhang et al., 2016). Berberine was found to enhance DNA methylation throughout the genome, but it inhibited expression of CAR and its target genes CYP2B6 and CYP3A4. Berberine altered the morphology of murine L929 cells and reduced cell cycle division at concentrations above 0.1 mg/ml (Gu et al., 2015). Cell viability

decreased *in vitro* at concentrations greater than 0.05 mg/ml and DNA damage reported at greater than 0.025 mg/ml. Significantly increased reactive oxygen species were found at the lowest concentration assayed (0.005 mg/ml). Wang et al., (2016) concluded that berberine acts as an epigenetic modulator affecting enzymes involved in histone acetylation and modulation. Concentrations associated with DNA damage are much greater than the plasma concentrations associated with therapeutic doses. As noted above in the ADME section, a single oral dose of 132 mg in rats resulted in a C_{max} of 1.1 ng/ml of berberine (Gupta et al., 2010).

Preclinical Safety Data Summary

Berberine has been assessed in acute, subchronic, chronic/carcinogenicity, genotoxicity, developmental and target animal safety studies as a component of goldenseal. These studies of the goldenseal product containing 3.45-3.89% berberine and the fibrous root of *Rhizoma Coptidis* containing 1.20% berberine provide indirect information regarding potential berberine toxicity. The studies can be used to assess berberine by assuming, as a worst case, that all of the toxicity in the studies is due to berberine. Other isoquinoline components of goldenseal, however, include hydrastine (2.0%) and canadine (0.5%) and these alkaloids are expected to contribute to goldenseal toxicity. Likewise, the fibrous root of *Rhizoma Coptidis* was determined to contain coptisine (1.28%), epiberberine (0.53%), and palmatine (0.08%) (Ning et al., 2015). A summary of the published safety data for berberine is provided in **Table 13** below. Assuming the highest 90th percentile intake among the children aged 2-5 years (9.1 E-6 mg/kg bw/day, see **Table 8**) and comparing to the subchronic NOAELs and LOAELs for berberine, the margin of exposures (MOE) are very large, > 100,000, see **Table 13**.

Comparison of the maternal NOAELs in developmental toxicity studies for berberine tested as a component of goldenseal and alone confirms the significant probability that other components of goldenseal contribute significantly to the toxicity of the mixture. Risk assessment based on the assumption that berberine content alone is responsible for the toxicological effects seen in studies of goldenseal greatly overestimates berberine risk.

Table 13. Toxicity studies Summary of berberine when tested as a component of the fibrous root of *Rhizoma Coptidis* and Goldenseal root powder[4]

Assay	**Berberine Source**	**Berberine Content (%)**	**Species**	**Effect**	**NOAEL for Berberine Content (mg/kg bw/day)**	**Reference**	**Margin of Exposure (MOE) Based on c2-5y 90th**
Subchronic	fibrous root of *Rhizoma Coptidis*	1.20	Rat	Liver and lung lesions, increased liver enzymes	22.56	Ning et al., 2015	2.5E+06
Subchronic	Goldenseal root powder	3.45	Rat	Clinical pathology	LOAEL = 9 (inferred)	NTP (2010)	9.9E+05
Subchronic	Goldenseal root powder	3.45	Mouse	Clinical pathology	47 (males) - 43(females) (inferred)	NTP (2010)	4.7E+06
Developmental	Goldenseal root powder	5	Rat	No developmental toxicity	61	NTP (2003)	4.7E+06
Developmental	Goldenseal root powder	5	Mouse	Decreased fetal weight	102	NTP (2003)	1.1E+07

[4] Berberine chloride dihydrate was tested in developmental toxicity studies in rats and mice. The NOAELs were 531 and 841 mg/kg bw/day, respectively (equivalent to 420 and 666 mg/kg bw/day of berberine chloride, respectively).

An increased incidence of hepatocellular tumors was found in male and female rats receiving diet containing goldenseal with a 3.89% concentration of berberine. IARC classified goldenseal root powder as "possibly carcinogenic to humans" (Group 2B). The tumor response was found in a single site (liver) in a single species (rat). Although one carcinoma was seen in one treated male rat at the high dose level, only benign tumors were clearly treatment-related.

Berberine is not mutagenic but it inhibits topoisomerase, a category of DNA repair enzymes. Berberine also has antiproliferative properties *in vitro*. Dietary exposure to the target species and humans is low. The tumor dose-response of goldenseal suggests nonlinearity and a threshold of 15.75 mg/kg bw/day or greater. Assuming the highest 90th percentile intake among children 2-5 years (9.1E-06 mg/kg bw/day, see **Table 8**), this threshold dose is 1,700,000-fold greater than the estimated dietary exposure from the consumption of chicken.

Although berberine may be considered associated with an increase in benign liver tumors in the rat when tested as a component of the goldenseal root powder, these effects may be secondary to changes in liver function or due to other components of the goldenseal mixture. The absence of berberine induced genotoxicity or developmental toxicity suggests that berberine is not a direct acting carcinogen[5].

Clinical Safety Data

Numerous clinical studies have been conducted with berberine or berberine-containing plant extracts. These studies, including systematic reviews and meta-analyses, largely focused on efficacy in areas pertaining to gastrointestinal effects, cardiovascular disorders, and metabolic disease. The PubMed literature search for clinical studies with berberine yielded >150 papers (the search string is detailed in **Table 9**). Many of the captured studies were based on topics such as efficacy, combination therapies and pharmacokinetics.

[5] The etiologies of developmental toxicity have many common pathways with carcinogenicity. For example, epigenetic changes induced *in utero* may increase susceptibility to cancer in aging animals (Ho et al., 2017; Cheong et al. 2017). Cytotoxicity and alterations in rates of cell division may be early events in both carcinogenicity and developmental toxicity.

Berberine has been shown to be safe in the majority of clinical trials; however, in a small percentage of patients, berberine has been reported to cause gastrointestinal reactions such as nausea, vomiting, constipation, diarrhea, abdominal distention, and abdominal pain (Mannarino et al., 2014; Lan et al., 2015; DeRosa et al., 2012), hypertension, respiratory failure and paresthesia (DeRosa et al., 2012). The most studied side effects are those on the gastrointestinal system. In fact, berberine and derivatives can produce gastric lesions (Cicerro et al, 2012). It has been shown that berberine delays small intestine transit time, which may account for part of its gastrointestinal effect (Cicerro et al., 2012). Berberine is also noted to increase gastric emptying time which could then potentially increase their gastrointestinal effects (Cicero et al., 2012).

Recently, several published systematic reviews and meta-analyses from 2018-2020 support previous reports of gastrointestinal-related adverse events (Cicero et al., 2018; Jianqing et al., 2018; Xie et al., 2019; Hu et al., 2020). While other systematic reviews of clinical studies evaluating berberine beneficial effects (improvement in fertility) in women with polycystic ovary syndrome (Mirzaee et al, 2020), therapeutic effects on metabolic diseases (Xu et al, 2020) and for diarrhea in children and adults (Yu et al, 2020) show no serious side effects and only mild gastrointestinal side effects. Standard doses of berberine are generally well tolerated and adverse reactions are rare and mild. On the contrary, high doses have been associated with arterial hypotension, dyspnoea, flu-like symptoms, gastrointestinal discomfort, constipation and cardiac damage. In a meta-analysis of 27 clinical trials on the effect and safety of berberine in the treatment of type 2 diabetes, hyperlipemia and hypertension, 12 trials reported a number of adverse reactions and eight others stated a slight adverse reaction without clear data. The statistical analysis showed that the incidence of toxic side effects is related to doses of berberine. At 600 mg berberine, the incidence rate of toxic side effects is 5%; when berberine dose is in the range of 900 to 1500 mg, the incidence rate is greater than 10% (as shown in **Figure 8** from Lan et al., 2015). The highest 90th percentile EDI for berberine from chicken consumption among children aged 2-5 years (0.00015 mg/day, see **Table 8**) is orders of magnitude below these observed adverse effect levels, and thus, not a safety concern.

Antimicrobial Resistance Risk

Hazard Characterization

There is a theoretical concern that the risk for antimicrobial resistance could develop among zoonotic, bacterial pathogens of human health concern associated with the proposed use of berberine as a preservative in poultry feed (FDA-CVM[6], 2020). As such, a hazard characterization following the FDA's GFI #152[7] was carried out to confirm that such potential risks do not exist with the proposed use of berberine.

The sources, chemistry, uptake and excretion of berberine was previously presented. Berberine is not a typical antimicrobial and does not belong in any standard antimicrobial groupings. Berberine has shown a range of antimicrobial mechanisms. It causes cell membrane damage, cell membrane permeability changes and efflux pump inhibition (Cao et al. 2016; Jeyakkumar et al., 2016; Karaosmanaglu et al., 2014; Peng et al., 2015; Zhang et al., 2020). Interference with efflux pumping was observed to increase the lethality of other antibacterial agents (Wojtyczka et al., 2014; Yu et al., 2006). Evidence was also presented that berberine affects DNA/RNA and protein synthesis (Amin et al., 1969; Cao et al. 2016; Jeyakkumar et al., 2016; Karaosmanaglu et al., 2014; Peng et al., 2015; Wu et al., 2005).

Berberine has a wide antimicrobial and antiprotozoal activity (Kumar et al., 2015). Literature reports activity against both Gram positive (MRSA) and Gram negative (*Escherichia coli*) (Jeyakkumar et al., 2016); Gram negative *Pseudomonas aeruginosa* and *Escherichia coli*, Gram positive *Bacillus subtilis* and *Staphylococcus aureus*; six filamentous fungi; and two yeasts (Cernakova and Kostalova, 2002); MRSA (Yu et al., 2006; Zhang et al., 2020); fourteen strains

[6] FDA CVM Meeting Minutes from the Feb 12, 2020 between CVM and IRP Health Pty. Ltd. The purpose of the meeting was to discuss pre-submission information regarding the use of berberine as a preservative in poultry feed.

[7] FDA #152, Guidance for Industry: Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern - provides guidance on a risk assessment approach that "focuses on the concern that the use of antimicrobial new animal drugs in food-producing animals will result in the emergence and selection of antimicrobial resistant food-borne bacteria which impact human health adversely." "The FDA believes that human exposure through the ingestion of antimicrobial resistant bacteria from animal-derived foods represents the most significant pathway for human exposure to bacteria that have emerged or been selected as a consequence of antimicrobial drug use in animals."

of *Staphylococcus aureus* (Wojtyczke et al., 2014); *Streptococcus agalactiae* (Peng et al., 2015); *Vibrio cholera* and *Staphylococcus aureus* (Amin et al., 1969); *Escherichia coli* and *Bacillus subtilis* (Kong et al., 2012); *Salmonella Typhi* (Wu et al., 2005); *Shigella dysenteriae* (Kong et al., 2010); and *Brucella abortus* (Azimi et al., 2018).

The ability of berberine to inhibit growth was determined against many bacteria. The effective concentrations (MIC, IC_{50}) are summarized below (**Table 14**). The antimicrobial effect on some molds and yeasts were also reported by Cernakova and Kostalova (2002).

Table 14. Inhibitory Concentrations of Berberine

Bacteria	**MIC (ug/mL)**	**Reference**
Streptococcus agalactiae	78	Peng et al., 2015
MRSA strains	32-128	Yu et al., 2006
$MRSA_{252}$	128	Zhang et al., 2020
Staphylococcus 14 coagulase negative strains *Staphylococcus aureus*	159 average (16-512 range) 212	Wojtyczka et al., 2014 Cernakova & Kostalova, 2002
Escherichia coli Sensitive strain Resistant strain	 230 469	Cernakova & Kostalova, 2002
Pseudomonas aeruginosa Sensitive strain Resistant strain	 226 240	Cernakova & Kostalova, 2002
	IC_{50} (ug/mL)	
Escherichia coli *Bacillus subtilis*	582 952	Kong et al., 2012
	Inhibitory concentration (ug/mL)	
Shigella dysenteriae	250-300	Kong et al., 2010
	MIC (ug/mL)	
6 Molds	997 average (584-1904 range)	Cernakova & Kostalova, 2002
Yeasts *Saccharomyces cerevisiae* *Candida albicans*	 1935 1576	Cernakova & Kostalova, 2002

Amin et al. (1969) reported berberine sulfate was bacteriocidal to *Vibrio cholerae* and bacteriostatic to *Staphylococcus aureus* at 35 and 50 ug/mL, respectively. Azimi et al. (2018) reported the MIC for berberine against *Brucella abortus* was 1.56 ug/mL and the minimum bactericidal concentration (MBC) was 3.12 ug/mL.

Berberine is not on FDA's antimicrobial drug list (FDA #152 Appendix A, Table A1). From a plant source, it is a traditional Chinese medicine and can be purchased in the U.S. as a nutritional supplement.

The primary human pathogens associated with poultry are *Salmonella* and *Campylobacte*r. Berberine is not a drug for treatment of these pathogens in humans. No studies were found showing the development of resistance to berberine by pathogenic bacteria. However, berberine has been shown to have a non-specific interaction with various cellular moieties in the membrane, nucleic acids and proteins which would not lead to development of specific antimicrobial resistance mechanisms.

Berberine Metabolites

As earlier summarized, berberine is metabolized in the liver, in both rats and humans, undergoing demethylation in Phase I metabolism, followed by conjugation with glucuronic acid or sulfuric acid to form Phase II metabolites (Kumar et al., 2015). Jatrorhizine-3-sulfate, demethylene berberine 2-sulfate and thalifendine-10-sulfate are 3 metabolites of berberine that have been identified in the urine of human volunteers (Pan et al., 2002).

The cytochrome P450 enzymes that have been identified in berberine metabolism are CYP2D, CYP1A2, and CYP3A4. The predominant cytochrome P450 enzyme involved in berberine metabolism is considered to be CYP2D (Guo et al., 2011; Habtemarium, 2020). While these studies were performed in mammalian species such as humans, mice, and rats, orthologous cytochrome P450 enzymes exist in chickens. Therefore, it is plausible that the metabolic pathway for berberine in chickens is similar to other metabolic pathways that have been identified in mammalian species (Murcia et al., 2011; Cai et al., 2012; Tan et al., 2013; Watanabe et al., 2013; Yang et al., 2013).

No information on the antimicrobial activity of the demethylene berberine 2-sulfate and thalifendine-10-sulfate metabolites was identified in the published literature. Wang et al. (2017) reviewed berberine metabolism and summarized several studies on the antimicrobial activity of the metabolite jatrorrhizine, including inhibition of the growth of *Staphylococcus aureus* in *in vitro* studies (Yu et al., 2007); broad spectrum of antimicrobial activity against *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidi*, *Micrococcus lysodeikticus*, *Proteus vulgaris*, *Salmonella typhi*, and *Escherichia coli*, with minimum inhibitory concentration (MIC) values of 0.1-0.6 g/l (Deng et al., 2012); and *in vitro* inhibitory activity against dermatophytes such as *Trichophyton rubum* and *Microsporum canis* (Vollekova et al., 2003). In another study conducted by Azini et al (2018), whereby the anti-brucellosis properties of four isolated alkaloid compounds (i.e., palmatine, berberine, columbamine, and jatrorrhizine) were studied against *B. abortus* under different test conditions. Jatrorrhizine was shown to have more antibacterial activity with MIC and MBC of 0.78 and 1.56 µg/mL, respectively.

Tissue Residue

There is a theoretical concern that berberine or its active metabolite may accumulate in edible animal tissues and upon ingestion may negatively impact the intestinal flora of human consumers (FDA-CVM, 2020, meeting minutes). As described in the study conducted by Haipeng et al. (2009), orally administered berberine was poorly absorbed in chickens and orally administered berberine yielded a low absolute bioavailability of 2.03%. Pharmacokinetic studies conducted in mammalian species demonstrate a similar metabolic profile of poor absorption following oral administration of berberine and low oral bioavailability (Wang et al, 2017; Xu et al., 2019; Habtemariam, 2020).

Assuming that the pharmacokinetics of berberine in chickens is similar to mammals, it is possible to refer to tissue distribution studies of berberine metabolites in mammals as a likely scenario in chickens. For example, in rats that were administered 200 mg/kg bw of berberine, the metabolites thalifendine (M1), berberrubine (M2), and jatrorrhizine (M4) were detected in tissues such as the liver, kidney, and lung, while demethyleneberberine (M3) was not detected in any of the tissues (i.e., liver, kidney, lung, heart, muscle, pancreas) that were evaluated (Tan et

al., 2013). The M1 and M4 metabolites distributed primarily to the liver and kidney, with minimal levels detected in the lung (Tan et al., 2013). Metabolite concentrations also peaked within 24 hours after dosing in all tissues (Tan et al., 2013). The study authors further concluded that the volume of berberine and its metabolite tissue distribution occurred in a descending order of berberine>M2>M1>>M4 (Tan et al., 2013). Separately, results from a chicken residues study revealed that berberine was not detected (LOD of 2 ng/g) in the liver and muscle tissues of chickens fed a low level of berberine in their diet (0.03g berberine/kg feed) (Yu et al., 2017) .

While Tan et al. (2013) showed that M1 and M4 metabolites distributed to the liver, kidney, and minimally to the lung, but were undetected in the muscle tissues of rats, it is worth noting that these tissues are not typically considered the major edible tissues of the chicken. A similar argument can be made for the parent compound berberine that was detected in the following rat tissues in descending order of abundance: liver>kidney>muscle>lung>brain>heart>pancreas>fat (Tan et al., 2013). Additionally, berberine distribution in the liver reached a maximum concentration 8 hours after berberine was administered and then rapidly declined, followed by a plateauing effect throughout the remainder of the study duration (i.e., 36 hours) (Tan et al., 2013). Taken together, berberine and its active metabolites are not expected to accumulate in the major edible tissues of the chicken. Furthermore, since berberine is poorly absorbed in chickens and is not detected in muscle or liver tissues, the metabolites are also not expected to be present in chicken muscle or tissues.

Safety Determination

The substance of this review is berberine, with purity of NLT 97.0% berberine (anhydrous basis). The chemical name is 5,6-Dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-α] quinolizinium chloride hydrate. It is extracted from dry bark of *Phellodendron chinense* with a hot solvent mixture of (b) (4) and (b) (4) and is produced in accordance with cGMP. Berberine meets appropriate animal food-grade specifications. Analytical data from non-consecutive batches demonstrate the product specifications are consistently met.

Berberine is intended for use as a preservative in feed for broiler chickens. It is proposed to be added to feed at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold.

Feed intake by poultry varies due to many factors such as size, age, gender, breed, environment, level of egg production, and quality of feed. Based on the proposed inclusion rate of berberine in feed of 0.03 g/kg (or 0.03 mg/g), the daily intake of berberine for male and female broilers are 6.12 mg/day and 4.95 mg/day, respectively.

Results from a poultry feeding study showed that at the "low" berberine diet concentration of 0.03 g/kg, the proposed maximum use level, berberine residues in chicken tissues were below the LOD of 2 ng/g. Assuming the maximum residue level in broiler chicken tissues at the LOD (2 ng/g) and intake of chicken food (muscle tissue and organs) based on U.S. NHANES 2013-2016 food consumption data, the 90th percentile EDI of berberine from the proposed use is 0.0000091 mg/kg bw/day and 0.0000039 mg/kg bw/day for children aged 2-5 y and for the US population aged 2+y, respectively.

Literature searches were conducted in December 2020 using PubMed, with supplemental searches performed in GoogleScholar to identify studies containing information pertinent to the safety of berberine. Additional databases that were searched included the NTP, EFSA, FDA, FSANZ, and JECFA.

Chicken Safety Data-- Orally administered berberine was poorly absorbed in chickens, as indicated by the low absolute bioavailability of 2.03% and Cmax of 4.09 µg/l. Intravenously administered berberine resulted in rapid absorption, distribution, and elimination of berberine. Berberine was not detected (LOD of 2 ng/g) in tissues of chickens fed a low level of berberine in their diet (0.03g berberine/kg feed). Feeding studies showed no safety concern in chickens fed with berberine containing feed at concentration up to 1 g/kg. IRP Health's proposed maximum use level of berberine in chicken feed is 0.03g/kg and is, therefore, safe for broiler chickens.

Human Safety Data -- Berberine has been assessed in acute, subchronic, chronic/carcinogenicity, genotoxicity, developmental and target animal safety studies as a component of goldenseal.

These studies of the goldenseal product containing 3.45-3.89% berberine and the fibrous root of *Rhizoma Coptidis* containing 1.20% berberine provide indirect information regarding potential berberine toxicity. The studies can be used to assess berberine by assuming, as a worst case, that all of the toxicity in the studies is due to berberine. Other isoquinoline components of goldenseal, however, include hydrastine (2.0%) and canadine (0.5%) and these alkaloids are expected to contribute to goldenseal toxicity. Likewise, the fibrous root of *Rhizoma Coptidis* was determined to contain coptisine (1.28%), epiberberine (0.53%), and palmatine (0.08%) (Ning et al., 2015). Based on the highest 90th percentile intake among children aged 2-5 years (9.1 E-6 mg/kg bw/day) and comparing to the available subchronic NOAELs and LOAELs for berberine, the MOE are very large, > 100,000.

Comparison of the maternal NOAELs in developmental toxicity studies for berberine tested as a component of goldenseal and alone confirms the significant probability that other components of goldenseal contribute significantly to the toxicity of the mixture. Risk assessment based on the assumption that berberine content alone is responsible for the toxicological effects seen in studies of goldenseal greatly overestimates berberine risk.

An increased incidence of hepatocellular tumors was found in male and female rats receiving diet containing goldenseal with a 3.89% concentration of berberine. IARC classified goldenseal root powder as "possibly carcinogenic to humans" (Group 2B). The tumor response was found in a single site (liver) in a single species (rat). Although one carcinoma was seen in one treated male rat at the high dose level, only benign tumors were clearly treatment-related.

Berberine is not mutagenic but it inhibits topoisomerase, a category of DNA repair enzymes. Berberine also has antiproliferative properties *in vitro*. Dietary exposure to the target species and humans is low. The tumor dose-response of goldenseal in rats suggests nonlinearity and a threshold of 15.75 mg/kg bw/day or greater. Based on the highest 90th percentile intake among the children aged 2-5 years (9.1E-06 mg/kg bw/day), this threshold dose is 1,700,000-fold greater than the estimated dietary exposure from the consumption of chicken.

Although berberine is associated with an increase in benign liver tumors in the rat when tested as a component of the goldenseal root powder, these effects may be secondary to changes in liver

function or due to other components of the goldenseal mixture. The absence of berberine induced genotoxicity or developmental toxicity suggests that berberine is not a direct acting carcinogen[8].

Recently published systematic reviews and meta-analyses from randomized control trials (2018-2020) support previous reports of gastrointestinal-related adverse events (Cicero et al., 2018; Jianqing et al., 2018; Xie et al., 2019; Hu et al., 2020). Standard doses of berberine are generally well tolerated and adverse reactions are rare and mild. On the contrary, high doses have been associated with arterial hypotension, dyspnoea, flu-like symptoms, gastrointestinal discomfort, constipation and cardiac damage. In a meta-analysis of 27 clinical trials on the effect and safety of berberine in the treatment of type 2 diabetes, hyperlipidemia and hypertension, 12 trials reported a number of adverse reactions and eight others stated slight adverse reactions without clear data. The statistical analysis showed that the incidence of toxic side effects is related to doses of berberine. At 600 mg berberine, the incidence rate of toxic side effects is 5%; when berberine dose is in the range of 900 to 1500 mg, the incidence rate is greater than 10% (Lan et al., 2015). The highest 90th percentile EDI for berberine from chicken consumption among children aged 2-5 years (0.00015 mg/day) is orders of magnitude below these observed adverse effect levels, and thus, not a safety concern.

Antimicrobial Resistance Risk -- A hazard characterization following the FDA's GFI #152 was carried out to consider the theoretical concern that antimicrobial resistance could develop among zoonotic, bacterial pathogens of human health concern associated with the proposed use of berberine as a preservative in poultry feed. Berberine is not a typical antimicrobial and does not belong in any standard antimicrobial groupings. Berberine has shown a range of antimicrobial mechanisms. It causes cell membrane damage, cell membrane permeability changes and efflux pump inhibition. Interference with efflux pumping was observed to increase the lethality of other antibacterial agents. Evidence was also presented that berberine affects DNA/RNA and protein synthesis. Literature reports activity against both Gram positive (MRSA) and Gram

[8] The etiologies of developmental toxicity have many common pathways with carcinogenicity. For example, epigenetic changes induced *in utero* may increase susceptibility to cancer in aging animals (Ho et al., 2017; Cheong et al. 2017). Cytotoxicity and alterations in rates of cell division may be early events in both carcinogenicity and developmental toxicity.

negative, and the ability of berberine to inhibit growth was determined against many bacteria. Berberine is not on FDA's antimicrobial drug list. The primary human pathogens associated with poultry are Salmonella and Campylobacter and berberine is not a treatment drug for humans for these pathogens. No studies were found showing the development of resistance to berberine by pathogenic bacteria. Furthermore, berberine appears to have a non-specific interaction with various cellular moieties in the membrane, nucleic acids and proteins and is, thus, not expected to lead to development of specific antimicrobial resistance mechanisms.

Conclusion: A critical evaluation of the publicly available information on berberine leads to the conclusion that the proposed use of berberine as a preservative in feed for broiler chickens at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold meets the FDA safety standard of a reasonable certainty of no harm under the conditions of intended use, and is therefore safe. The pivotal data and information upon which this conclusion is based are publicly available, and generally known and generally accepted by the broader scientific community of experts qualified by experience and training to evaluate the safety of food ingredients for use in human food and animal feed. Thus, the intended use of berberine as a preservative in feed for broiler chickens as described is also GRAS, based on scientific procedures.

Discussion of Information Inconsistent with GRAS Determination

No information has been identified that would be inconsistent with a finding that the proposed use of berberine as a preservative in feed for broiler chickens to preserve the feed from yeast and mold, meeting appropriate specifications specified herein and used according to cGMP, is safe and GRAS based on scientific procedures, under the conditions of intended use in foods.

Basis for Conclusion that there is Consensus Regarding Safety

General recognition of safety through scientific procedures requires common knowledge throughout the scientific community knowledgeable about the safety of food ingredients that there is a reasonable certainty that a substance is not harmful under the intended conditions of use in foods. The aforementioned regulatory and scientific reviews related to safety of berberine

are published in the scientific literature and, therefore, are generally available and generally known among the community of qualified food ingredient safety experts. There is broad-based and widely disseminated knowledge concerning berberine. The data and publicly available information supporting the safety of the proposed use of berberine as proposed in this notification are not only widely known and disseminated, but are also commonly accepted among qualified food safety experts.

The GRAS Panel consists of the following qualified experts: William Allaben, Ph.D., ATS (Adjunct Professor, College of Medicine, College of Public Health, University of Arkansas for Medical Sciences; Toxicology Advisors, LLC), William Dozier, Ph.D. (Professor, Department of Poultry Science, College of Agriculture, Auburn University), and Michael W. Pariza, Ph.D. (Professor and Director Emeritus, Food Research Institute, University of Wisconsin Madison; Michael W. Pariza, Consulting, LLC). The GRAS Panel critically evaluated Exponent's "Documentation Supporting the Generally Recognized as Safe (GRAS) Status of the Use of Berberine in Feed for Broiler Chickens" as well as any other available data and information that the members of the GRAS Panel believed to be pertinent to the safety of the proposed use of berberine.

The GRAS Panel convened on September 28, 2020 via teleconference and independently, jointly, and unanimously concluded that IRP Health's berberine produced consistent with current good manufacturing practice (cGMP) and meeting the stated specifications, is safe for use as a preservative in feed for broiler chickens at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold. The GRAS Panel further concluded unanimously that the intended use of IRP Health's berberine is GRAS based on scientific procedures. It is also the unanimous consensus opinion of this GRAS Panel that other qualified experts would concur with these conclusions. The GRAS Panel Signed Consensus Statement is located in **Appendix I.**

The intended use of use of berberine as a preservative in feed for broiler chickens at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold has been determined to be safe through scientific procedures as set forth in 21 CFR§570.30(b), thus satisfying the so-called "technical" element of the GRAS determination. Because this safety

evaluation was based on generally available and widely accepted data and information, it also satisfies the so-called “common knowledge” element of a GRAS determination.

Part 7. List of supporting data and information (21 CFR § 570.255)

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APPENDICES

Appendix	Title
A	Raw Bark Quality Control Standard
B	Identification and Method of Assay
C	Pesticides Analytical Results
D	Dioxin Analytical Results
E	PCBs Analytical Results
F	Stability Study Reports: 1) in storage 2) in mash and pellets for broiler chickens
G	Efficacy Study Report
H	FNDDS Ingredients with Chicken-Derived Components Used in the Analysis of EDIs
I	Signed GRAS Panel Statement

Appendix A. Raw Bark Quality Control Standard

Raw Bark Quality Control Standard

English Translation

(b) (4)

(b) (4)

(b) (4)

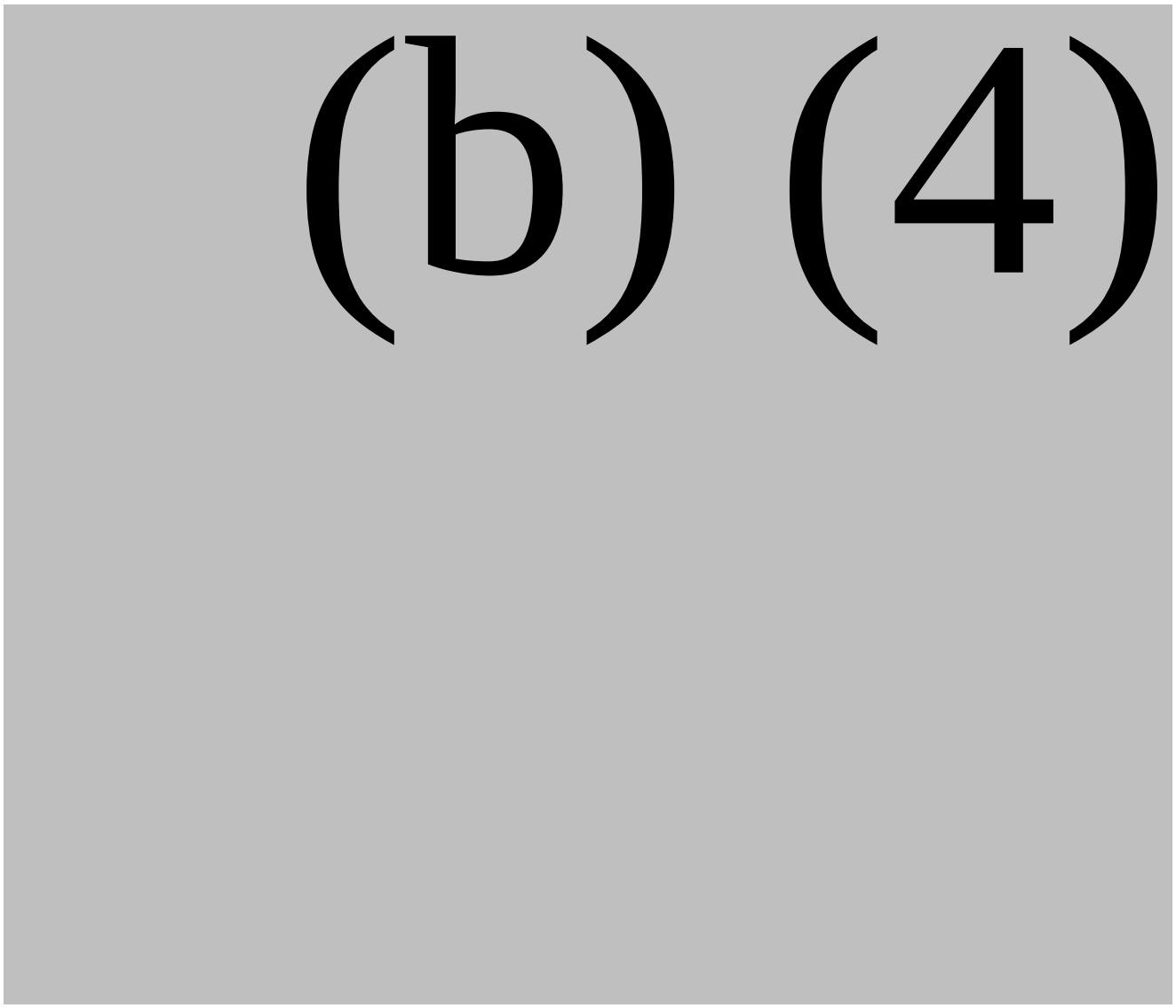
Name of document			Code of document		Page No.
Quality Standard of Raw Materials for Cortex Phellodendri			(b) (4)		Page 1 of 2
Drafted by/Date	(b) (6) 18 August 2016	Reviewed by/Date	(b) (6) 18 August 2016	QA reviewed by/Date	(b) (6) (illegible) 19 August 2016
Approved by	(signature illegible)	Date of approval	22 August 2016	Date of implementation	01 September 2016
Issuing department	Quality Assurance Department				
Distributed to	Quality Inspection Department, Quality Assurance Department, Logistics Department, and Production Department				

(Seal: Controlled document)

I. Purpose: Establish the quality standard of raw materials for cortex phellodendri, so as to control the quality of the raw materials under this Standard.

(b) (4)

(b) (4)



(b) (4)

Item	Control indicators	Testing method
* Characteristics	(b) (4)	Sensual examination
Identification		(b) (4)
(b) (4)		
Total ash		
Lead (Pb)		

(b) (4)

(b) (4)

Name of document	Quality Standard of Raw Materials for Cortex Phellodendri		
Code of document	(b) (4)	Page No.	Page 2 of 2
Arsenic (As)	≤ 2.0mg/kg		
Cadmium (Cd)	≤ 1.0mg/kg		
Mercury (Hg)	≤ 0.1mg/kg		
* Content proportion	berberine hydrochloride ⩾3.0%		(illegible)

(Seal: "Effective document, no copying permitted")

5.

6.

7.

(b) (4)

Serial No.	Date of change	Code of original document	Reasons for and content of change	Approved by
1	1 September 2016	(b) (4)	Version update	(b) (6)

(b) (4)

(b) (4)

Laboratory of (b) (4)

Certificate of Analysis

(Seal)

Serial No. of Testing: (b) (4) Report Date 24 October 2016

Name of sample	Cortex phellodendri	Batch No. of the product		(b) (4)
Department requesting testing	Logistics Department	Date of testing		20 October, 2018
Quantity of sample	(b) (4)	Status of sample		(b) (4)
Testing items	Method of analysis	Requirement of the standards	Result	Decision on the item
Identification	TLC	Spots of the same colour as those in a corresponding position in a chromatogram of the reference substance found.	Spots of the same color found	Requirement met
Characteristics	Visual inspection	Should be consistent with the standard	Consistent	Requirement met

(b) (4)

		characteristics of the raw material		
(b) (4)	(b) (4)			Requirement met
Total ash				Requirement met
Pb				Requirement met
As				Requirement met
Cd				Requirement met
Hg				Requirement met
Content proportion				Requirement met

(b) (4)

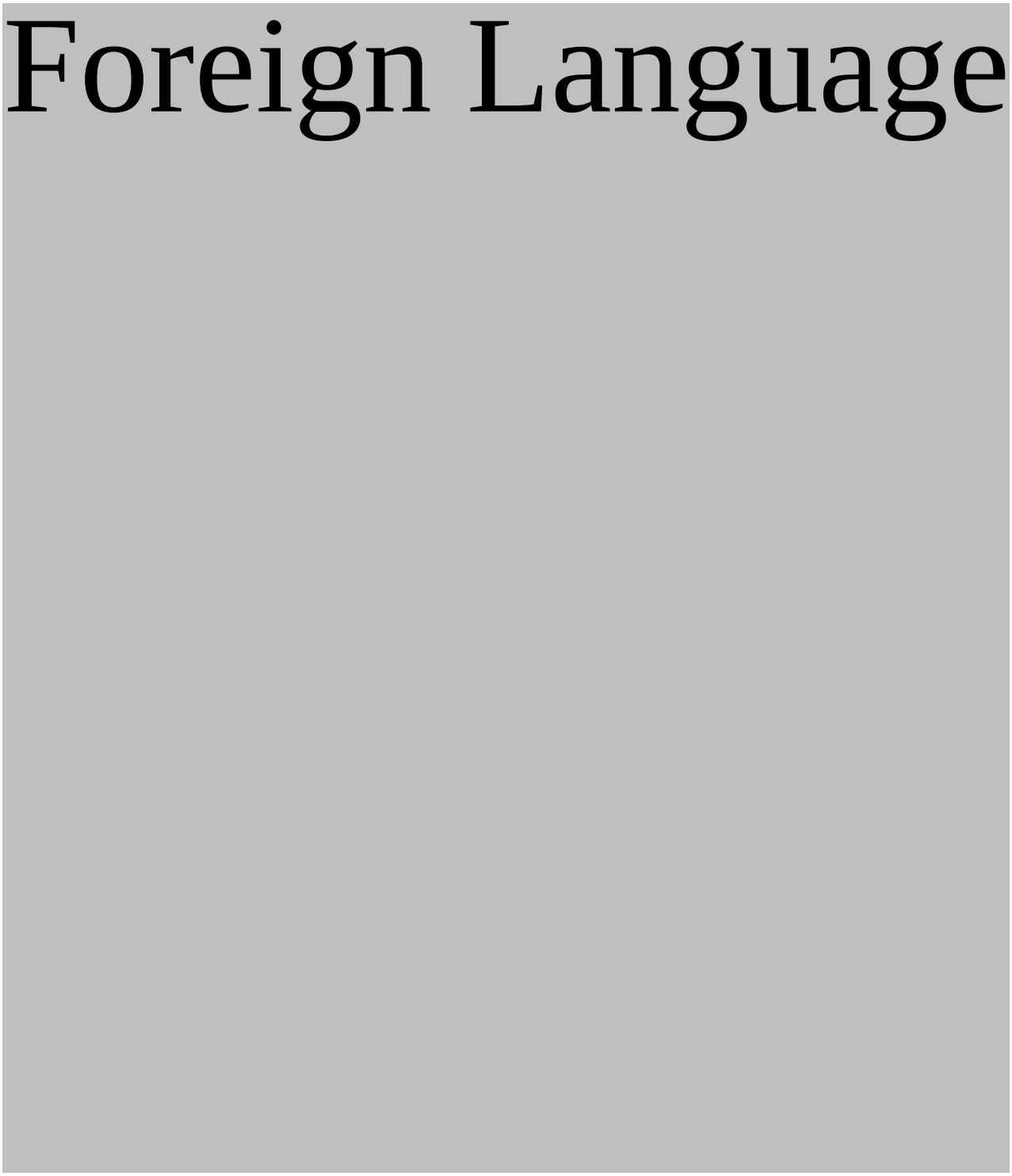
	cortex phellodendri			
Conclusion	Indicators tested for the batch of raw materials have met the requirements of the Standard.			
(Blank below)				

Approved by (b) (6) Checked by: (b) (6) Chief Inspector: (illegible)

Page 1 of 1

(b) (4)

Original Chinese Language



Foreign Language

Foreign Language

Appendix B. Identification and Method of Assay

JH/ZY-D-101.00’ No: 00; Revision No：01	
Approval date：	2010.07.05
Execute Date：	2010.07.05

Identification of 97% Berberine Hydrochloride (HPLC) extracted from dry bark of *Phellodenron chinensis* by HPTLC

1. OBJECTIVE

To identify 97% Berberine Hydrochloride (HPLC) extracted from dry bark of *Phellodendron chinensis* by HPTLC

2. SCOPE

This method is applied to identify 97% Berberine Hydrochloride (HPLC) extracted from dry bark of *Phellodendron chinensis*.

3. STANDARDS

Standard of berberine hydrochloride (National Institutes for Food and Drug Control)

4. EQUIPMENT

(b) (4)

5. PREPARATION OF SOLUTIONS

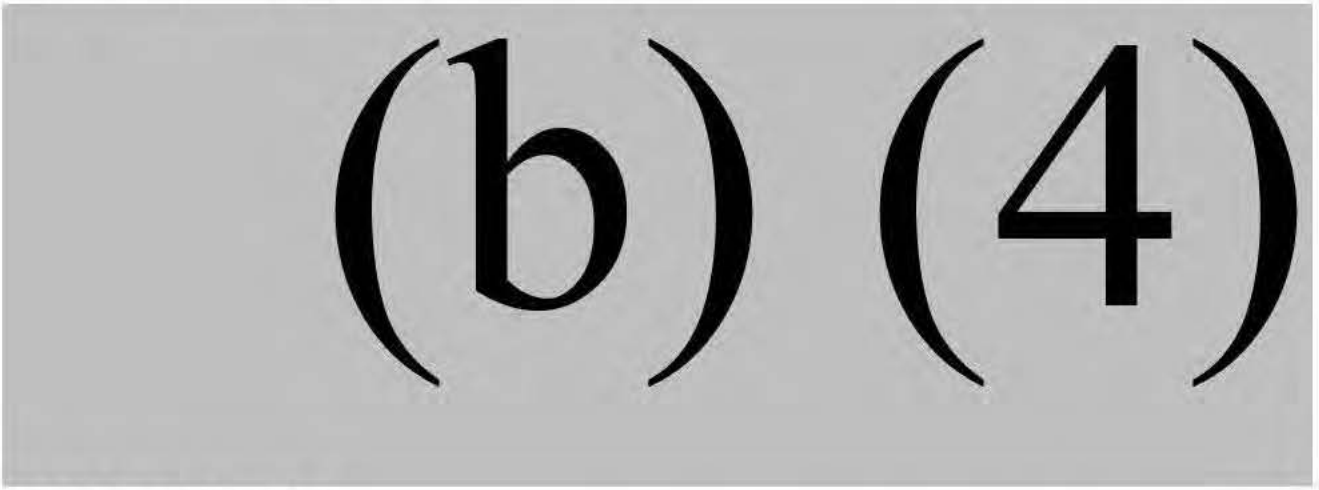
5.1 Preparation of standard solution

(b) (4).

5.2 Preparation of test sample solution:

(b) (4)

6. CHROMATOGRAPHIC CONDITION



7. SAMPLE TESTING

Accurately sampling the standard and test sample solutions on the same plate, develop the plate under the chromatographic conditions.

No. JH/ZY-C-143.00	
Approv date ：	2010.07.05
Execute Date:	2010.07.05

Determination of the content of Berberine Hydrochloride in the extract from dry bark of *Phellodendron chinensis* by HPLC

1. OBJECTIVE

To determinate the content of berberine hydrochloride in the extract from dry bark of *Phellodendron chinensis* by HPLC.

2. SCOPE

This method is applied to determine the content of berberine hydrochloride in the extract from dry bark of *Phellodendron chinensis.*

3. STANDARDS

Berberine Hydrochloride (National Institutes for Food and Drug Control)

4. REAGENTS

4.1 Methanol (analytic grade)

4.2 Acetonitrile (chromatographic grade)

4.3 Water (distilled water)

4.4 Phosphoric acid (analytic grade)

4.5 Dodecyl sulfonic acid sodium salt (analytic grade)

5. EQUIPMENT

5.1 HPLC with UV detector

5.2 Analytical balance (accurately to 0.01mg)

5.3 10-mL, 50-ml volumetric flask etc.

6. PREPARATION OF SOLUTIONS

6.1 **Preparation of mobile phase solution:**

(b) (4)

6.2 **Preparation of standard solution:**

(b) (4)

6.3 **Preparation of sample solution**

(b) (4)

7. CHROMATOGRAPHIC CONDITION

(b) (4)

8.SAMPLE TESTING

(b) (4)

9. CALCULATION

Calculate the percentage of berberine hydrochloride taken by the formula:

$$\text{Content}(\%)=\frac{A_1\times W_0\times V_1\times K}{A_0\times V_0\times W_1\times(1-d)}\times 100$$

In which:

A_1------- The peak area of berberine hydrochloride in the sample solution

A_0------- The peak area of berberine hydrochloride in the standard solution

W_1------- The weigh of sample (mg)

W_0------ The weigh of reference standard (mg)

V_1------- The total volume of sample solution (mL)

V_0------ The total volume of standard solution (mL)

K-------The purity of standard reference

d-------The (b) (4) of sample(%)

Appendix C. Pesticide Analytical Results

Appendix D. (b) (4) Pesticides Analytical Testing Results (mg/kg)

Pesticides (USP561); CPG Sec. 575.100 Pesticide Residues	Batch No.		
	IRP001 (CHI-A-910527)	CYSXBJ-C-A091253	CYSXBJ-C-A091398
Acephate	(b) (4)		
alachlor			
ALDRIN & DIELDRIN			
Azinphos-ethyl			
azinphos-methyl			
Bromide, inorganic (calculated as bromide ion)			
Bromophos-ethyl			
Bromophos-methyl			
Bromopropylate			
CHLORDANE			
CHLORDECONE (KEPONE)			
Chlorfenvinphos			
Chlorpyriphos-ethyl			
Chlorthal-dimethyl			
Cyfluthrin (sum of)			
Cyhalothrin -Lambda			
Cypermethrin and isomers (sum of)			
DDT, DDE, & TDE			
Deltamethrin			
Diazinon			
Dichlofluanid			
Dichlorvos			
DICOFOL (KELTHANE)			
Dimethoate and omethoate (sum of)			
Dithiocarbamates (expressed as CS2)			
Endosulfan (sum of isomers and endosulfan sulphate)			
Endrin			
Ethion			
ETHYLENE DIBROMIDE (EDB)			
Etrimfos			
Fenchlorophos (sum of fenchlorophos and fenchlorophos-oxon)			
Fenitrothion			
Fenpropathrin			
Fensulfothion			
Fenthion			
Fenvalerate			
Flucythrinate			
Fluvalinate – tau			
Fonofos			
HEPTACHLOR & HEPTACHLOR EPOXIDE			

(b) (4)

(b) (4)

(b) (4)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO Number: (b) (4)
Received On: 03Sep2020
Reported On: 10Dec2020

(b) (4) **Sample Code:** 468-2020-09030198
Client Sample Code: CHL-A-910527
Sample Description: plant extracted compound (97%)

Sample Registration Date: 03Sep2020
Condition Upon Receipt: acceptable, 25°C
Sample Reference:

QA0SY - Bromide, inorganic (GC-MS) <USP 561>	Reference	Accreditation	Completed
	EURL-SRM, Bromine Containing Fumigants	(b) (4)	10Sep2020

Parameter	Result
Total Inorganic Bromide	(b) (4)

QA0SW - Dithiocarbamates by LC-MS/MS per USP <561>	Reference	Accreditation	Completed
	Anal Bioanal Chem, 2008,392(5), 969-978.Modified.	(b) (4)	10Sep2020

Parameter	Result
Dithiocarbamates (as CS2)	(b) (4)

QA0SZ - Pesticides <USP 561> (LC-MS/MS and GC-MS/MS)	Reference	Accreditation	Completed
	USP 42 - 561 (Modified)	(b) (4)	21Oct2020

Parameter	Result
Acephate	(b) (4)
Alachlor	
Aldrin and dieldrin (sum of)	
Azinphos-ethyl	
Azinphos-methyl	
Bromophos-ethyl	
Bromophos-methyl	
Bromopropylate	
Chlordane (sum)	
Chlorfenvinphos	
Chlorpyrifos	
Chlorpyrifos-methyl	
Chlorthal-dimethyl	
Cyfluthrin I-IV	

(b) (4)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO Number: (b) (4)
Received On: 03Sep2020
Reported On: 10Dec2020

(b) (4) **Sample Code:** 468-2020-09030198
Client Sample Code: CHL-A-910527
Sample Description: plant extracted compound (97%)
Sample Registration Date: 03Sep2020
Condition Upon Receipt: acceptable, 25°C
Sample Reference:

QA0SZ - Pesticides <USP 561> (LC-MS/MS and GC-MS/MS)	Reference	Accreditation	Completed
	USP 42 - 561 (Modified)	(b) (4)	21Oct2020

Parameter	Result
Cyhalothrin, lambda-	(b) (4)
Cypermethrin I-IV	
DDT (sum)	
Deltamethrin	
Diazinon	
Dichlofluanid	
Dichlorvos	
Dicofol, o,p' & p,p'-	
Dimethoate and omethoate (sum of)	
Endosulfan (sum)	
Endrin	
Ethion	
Etrimfos	
Fenchlorophos (sum)	
Fenitrothion	
Fenpropathrin	
Fensulfothion (sum)	
Fenthion (sum)	
Fenvalerate I&II	
Flucythrinate I&II	
Fluvalinate, tau- I&II	
Fonofos	
Heptachlor (sum)	
Hexachlorobenzene (HCB)	
Hexachlorocyclohexane isomers (other than gamma)	
Lindane (gamma-HCH)	
Malathion and malaoxon (sum of)	
Mecarbam	
Methacrifos	
Methamidophos	
Methidathion	
Methoxychlor	
Mirex	
Monocrotophos	

(b) (4)

ANALYTICAL REPORT

(b) (4)
Report Supersedes (b) (4)

Client Code: (b) (4)
PO Number: (b) (4)
Received On: 03Sep2020
Reported On: 10Dec2020

(b) (4) **Sample Code:** 468-2020-09030198
Client Sample Code: CHL-A-910527
Sample Description: plant extracted compound (97%)
Sample Registration Date: 03Sep2020
Condition Upon Receipt: acceptable, 25°C
Sample Reference:

QA0SZ - Pesticides <USP 561> (LC-MS/MS and GC-MS/MS)	Reference	Accreditation	Completed
	USP 42 - 561 (Modified)	(b) (4)	21Oct2020

Parameter	Result
Parathion-ethyl and paraoxon-ethyl (sum of)	(b) (4)
Parathion-methyl and paraoxon-methyl (sum of)	
Pendimethalin	
Pentachloranisole	
Permethrin (sum of isomers)	
Phosalone	
Phosmet	
Piperonyl butoxide	
Pirimiphos-ethyl	
Pirimiphos-methyl (sum)	
Procymidone	
Profenofos	
Prothiofos	
Pyrethrum (total isomers)	
Quinalphos	
Quintozene (sum)	
S 421	
Tecnazene	
Tetradifon	
Vinclozolin	

QA0T0 - Standard Addition Confirmation <USP561>	Reference	Accreditation	Completed
	USP 42 - 561 (Modified)	(b) (4)	21Oct2020

Parameter	Result
Pentachloranisole	(b) (4)

Below USP limit for pentachlorani ole (0 01 mg/kg)

QA751 - Chlordecone	Reference	Completed
	USP 42 - 561 (Modified)	21Oct2020

Parameter	Result
Chlordecone (Kepone)	(b) (4)

QA524 - Ethylene dibromide (HS-GC-MS)	Reference	Completed
	USP 42 - 561 (Modified)	02Oct2020

(b) (4)

ANALYTICAL REPORT

Report Supersedes (b) (4)(b) (4)

Client Code: (b) (4)
PO Number: (b) (4)

Received On: 03Sep2020
Reported On: 10Dec2020

(b) (4) **Sample Code:**	468-2020-09030198	**Sample Registration Date:**	03Sep2020
Client Sample Code:	CHL-A-910527	**Condition Upon Receipt:**	acceptable, 25°C
Sample Description:	plant extracted compound (97%)	**Sample Reference:**	

QA524 - Ethylene dibromide (HS-GC-MS) **Reference** USP 42 - 561 (Modified) **Completed** 02Oct2020

Parameter	Result
Ethylene di-bromide	(b) (4)

Comments:
Rev.01: QA751 Chlordecone added per client request.
Rev.02: Client Sample Code updated per client request.
Rev.03: QA751 retested per client request.
Rev.04: Method reference updated per client request.

Respectfully Submitted,

(b)(6)

(b)(6)
Analytical Service Manager

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Client Code: (b) (4)
PO Number: waiting for the new quotation

ANALYTICAL REPORT

Received On: 25Sep2020
Reported On: 10Dec2020

(b) (4)
Report Supersedes (b) (4)

(b) (4) **Sample Code:** 468-2020-09250472
Client Sample Code: CYSXBJ-C-A091253
Sample Description: plant extracted compound (97%)
Sample Registration Date: 25Sep2020
Condition Upon Receipt: acceptable, 25°C
Sample Reference:

QA524 - Ethylene dibromide (HS-GC-MS)	**Reference** Internal		**Completed** 02Oct2020
Parameter Ethylene di-bromide	**Result** (b) (4)		
QA0SY - Bromide, inorganic (GC-MS) <USP 561>	**Reference** EURL-SRM, Bromine Containing Fumigants	**Accreditation** (b) (4)	**Completed** 02Oct2020
Parameter Total Inorganic Bromide	**Result** (b) (4)		
QA0SW - Dithiocarbamates by LC-MS/MS per USP <561>	**Reference** Anal Bioanal Chem, 2008,392(5), 969-978.Modified.	**Accreditation** (b) (4)	**Completed** 05Oct2020
Parameter Dithiocarbamates (as CS2)	**Result** (b) (4)		
QA751 - Chlordecone	**Reference** No Reference		**Completed** 21Oct2020
Parameter Chlordecone (Kepone)	**Result** (b) (4)		
QA0SZ - Pesticides <USP 561> (LC-MS/MS and GC-MS/MS)	**Reference** USP 42 - 561 (Modified)	(b) (4)	**Completed** 27Oct2020
Parameter Acephate Alachlor Aldrin and dieldrin (sum of) Azinphos-ethyl Azinphos-methyl	**Result** (b) (4)		

(b) (4)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO Number: waiting for the new quotation
Received On: 25Sep2020
Reported On: 10Dec2020

(b) (4) **Sample Code:** 468-2020-09250472
Client Sample Code: CYSXBJ-C-A091253
Sample Description: plant extracted compound (97%)
Sample Registration Date: 25Sep2020
Condition Upon Receipt: acceptable, 25°C
Sample Reference:

QA0SZ - Pesticides <USP 561> (LC-MS/MS and GC-MS/MS)	Reference	Accreditation	Completed
	USP 42 - 561 (Modified)	(b) (4)	27Oct2020

Parameter	Result
Bromophos-ethyl	(b) (4)
Bromophos-methyl	
Bromopropylate	
Chlordane (sum)	
Chlorfenvinphos	
Chlorpyrifos	
Chlorpyrifos-methyl	
Chlorthal-dimethyl	
Cyfluthrin I-IV	
Cyhalothrin, lambda-	
Cypermethrin I-IV	
DDT (sum)	
Deltamethrin	
Diazinon	
Dichlofluanid	
Dichlorvos	
Dicofol, o,p' & p,p'-	
Dimethoate and omethoate (sum of)	
Endosulfan (sum)	
Endrin	
Ethion	
Etrimfos	
Fenchlorophos (sum)	
Fenitrothion	
Fenpropathrin	
Fensulfothion (sum)	
Fenthion (sum)	
Fenvalerate I&II	
Flucythrinate I&II	
Fluvalinate, tau- I&II	
Fonofos	
Heptachlor (sum)	
Hexachlorobenzene (HCB)	
Hexachlorocyclohexane isomers (other than gamma)	

(b) (4)

ANALYTICAL REPORT

Client Code: (b) (4)

Received On: 25Sep2020
Reported On: 10Dec2020

(b) (4)
Report Supersedes (b) (4)

(b) (4) **Sample Code:** 468-2020-09250472
Client Sample Code: CYSXBJ-C-A091253
Sample Description: plant extracted compound (97%)

Sample Registration Date: 25Sep2020
Condition Upon Receipt: acceptable, 25°C
Sample Reference:

QA0SZ - Pesticides <USP 561> (LC-MS/MS and GC-MS/MS)	Reference	(b) (4)	Completed
	USP 42 - 561 (Modified)		27Oct2020

Parameter	Result
Lindane (gamma-HCH)	(b) (4)
Malathion and malaoxon (sum of)	
Mecarbam	
Methacrifos	
Methamidophos	
Methidathion	
Methoxychlor	
Mirex	
Monocrotophos	
Parathion-ethyl and paraoxon-ethyl (sum of)	
Parathion-methyl and paraoxon-methyl (sum of)	
Pendimethalin	
Pentachloranisole	
Permethrin (sum of isomers)	
Phosalone	
Phosmet	
Piperonyl butoxide	
Pirimiphos-ethyl	
Pirimiphos-methyl (sum)	
Procymidone	
Profenofos	
Prothiofos	
Pyrethrum (total isomers)	
Quinalphos	
Quintozene (sum)	
S 421	
Tecnazene	
Tetradifon	
Vinclozolin	

Comments:
Rev 01: Chlordecone and fluvalinate re-tested per client request.
Rev.02: Method reference updated per client request.

(b) (4)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO Number: waiting for the new quotation

Received On: 25Sep2020
Reported On: 10Dec2020

Respectfully Submitted,

(b)(6)

(b)(6)
Analytical Service Manager

(b) (4)

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ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO Number: waiting for the new quotation
Received On: 25Sep2020
Reported On: 10Dec2020

(b) (4) **Sample Code:** 468-2020-09250473
Client Sample Code: CYSXBJ-C-A091398
Sample Description: plant extracted compound (97%)
Sample Registration Date: 25Sep2020
Condition Upon Receipt: acceptable, 25°C
Sample Reference:

QA524 - Ethylene dibromide (HS-GC-MS)	**Reference** Internal		**Completed** 02Oct2020
Parameter Ethylene di-bromide	**Result** (b) (4)		
QA0SY - Bromide, inorganic (GC-MS) <USP 561>	**Reference** EURL-SRM, Bromine Containing Fumigants	**Accreditation** (b) (4)	**Completed** 05Oct2020
Parameter Total Inorganic Bromide	**Result** (b) (4)		
QA0SW - Dithiocarbamates by LC-MS/MS per USP <561>	**Reference** Anal Bioanal Chem, 2008,392(5), 969-978.Modified.	(b) (4)	**Completed** 05Oct2020
Parameter Dithiocarbamates (as CS2)	**Result** (b) (4)		
QA751 - Chlordecone	**Reference** No Reference		**Completed** 21Oct2020
Parameter Chlordecone (Kepone)	**Result** (b) (4)		
QA0SZ - Pesticides <USP 561> (LC-MS/MS and GC-MS/MS)	**Reference** USP 42 - 561 (Modified)	**Accreditation** (b) (4)	**Completed** 27Oct2020
Parameter Acephate Alachlor Aldrin and dieldrin (sum of) Azinphos-ethyl Azinphos-methyl	(b) (4)		

(b) (4)

ANALYTICAL REPORT

Report Supersedes (b) (4) (b) (4)

Client Code: (b) (4)
PO Number: waiting for the new quotation
Received On: 25Sep2020
Reported On: 10Dec2020

(b) (4) **Sample Code:** 468-2020-09250473
Client Sample Code: CYSXBJ-C-A091398
Sample Description: plant extracted compound (97%)
Sample Registration Date: 25Sep2020
Condition Upon Receipt: acceptable, 25°C
Sample Reference:

QA0SZ - Pesticides <USP 561> (LC-MS/MS and GC-MS/MS)	Reference	Accreditation	Completed
	USP 42 - 561 (Modified)	(b) (4)	27Oct2020

Parameter	Result
Bromophos-ethyl	(b) (4)
Bromophos-methyl	
Bromopropylate	
Chlordane (sum)	
Chlorfenvinphos	
Chlorpyrifos	
Chlorpyrifos-methyl	
Chlorthal-dimethyl	
Cyfluthrin I-IV	
Cyhalothrin, lambda-	
Cypermethrin I-IV	
DDT (sum)	
Deltamethrin	
Diazinon	
Dichlofluanid	
Dichlorvos	
Dicofol, o,p' & p,p'-	
Dimethoate and omethoate (sum of)	
Endosulfan (sum)	
Endrin	
Ethion	
Etrimfos	
Fenchlorophos (sum)	
Fenitrothion	
Fenpropathrin	
Fensulfothion (sum)	
Fenthion (sum)	
Fenvalerate I&II	
Flucythrinate I&II	
Fluvalinate, tau- I&II	
Fonofos	
Heptachlor (sum)	
Hexachlorobenzene (HCB)	
Hexachlorocyclohexane isomers (other than gamma)	

(b) (4)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO Number: waiting for the new quotation
Received On: 25Sep2020
Reported On: 10Dec2020

(b) (4) **Sample Code:** 468-2020-09250473
Client Sample Code: CYSXBJ-C-A091398
Sample Description: plant extracted compound (97%)
Sample Registration Date: 25Sep2020
Condition Upon Receipt: acceptable, 25°C
Sample Reference:

QA0SZ - Pesticides <USP 561> (LC-MS/MS and GC-MS/MS)
Reference USP 42 - 561 (Modified)
(b) (4)
Completed 27Oct2020

Parameter	Result
Lindane (gamma-HCH)	(b) (4)
Malathion and malaoxon (sum of)	
Mecarbam	
Methacrifos	
Methamidophos	
Methidathion	
Methoxychlor	
Mirex	
Monocrotophos	
Parathion-ethyl and paraoxon-ethyl (sum of)	
Parathion-methyl and paraoxon-methyl (sum of)	
Pendimethalin	
Pentachloranisole	
Permethrin (sum of isomers)	
Phosalone	
Phosmet	
Piperonyl butoxide	
Pirimiphos-ethyl	
Pirimiphos-methyl (sum)	
Procymidone	
Profenofos	
Prothiofos	
Pyrethrum (total isomers)	
Quinalphos	
Quintozene (sum)	
S 421	
Tecnazene	
Tetradifon	
Vinclozolin	

Comments:
Rev 01: Chlordecone and fluvalinate re-tested per client request.
Rev.02: Method reference updated per client request.

(b) (4)

ANALYTICAL REPORT

Report Supersedes (b) (4)

Client Code: (b) (4)
PO Number: waiting for the new quotation

Received On: 25Sep2020
Reported On: 10Dec2020

Respectfully Submitted,

(b)(6)

(b)(6)
Analytical Service Manager

(b) (4)

Appendix D. Dioxin Analytical Results

Dioxin Testing for Extracted Berberine

Dioxin testing of an extracted berberine sample for 17 dioxin like compounds (DLC) congeners, including 7 polychlorinated dibenzodioxins compounds and 10 polychlorinated dibenzofuran, were non-detected, except for 4 congeners: 1,2,3,4,6,7,8-HpCDF, OCDF, 1,2,3,4,6,7,8-HpCDD, and OCDD, with reported detected levels of 0.0484, 0.0732, 0.246, and 0.738 ng/kg, respectively. To appropriately interpret the results, the following comparisons were conducted:

(i) Compare the testing results with levels reported by US Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) in samples of poultry feed,

(ii) Estimate the potential dioxin levels in chicken meat that could result from feeding chicken the berberine product and compare these estimates to levels detected by the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) and FDA in chicken samples collected in the US.

In the first comparison, the 0.003 ng/kg (lower bound) and 0.029 ng/kg (medium bound) total 1998-WHO TEQ[1] levels in the berberine extract are lower than the 0.02 ng/kg and 3.35 ng/kg (ND=0) and 0.13 ng/kg and 3.38 ng/kg (ND=½ LOD) total 1998-TEQ levels reported in the deodorizer distillate and molasses, corn oil, and canola meal samples. The 4 congeners with detection in the berberine sample (1,2,3,4,6,7,8-HpCDF, OCDF, 1,2,3,4,6,7,8-HpCDD, and OCDD) are also the congeners that have the highest reported mean values for plant based animal feed in the FDA CVM survey, and the levels in the berberine product are well below the mean levels reported in the CVM samples for these 4 congeners.

In the second comparison, the calculated 1998-WHO total TEQ dioxin levels in chicken associated with the berberine product are comparable to the range of levels reported for chicken food samples in the FDA-TDS database and for young chicken in USDA FSIS database. The calculated 2005-WHO total TEQ dioxin level in chicken fat are within the ranges detected in the FDA-TDS ad USDA FSIS databases.

The sampling results and comparisons are detailed herein.

A. Testing Results

The laboratory test result is summarized in Table 1, and the full laboratory report in provided in **Attachment A**. The test, conducted using method GB 5009.205-2013, reports on the detected levels of 17 dioxin like compounds (DLC) congeners, including 7 polychlorinated dibenzodioxins compounds and 10 polychlorinated dibenzofuran compounds. The reported levels for all but 4 congeners were reported as less than the limit of reporting. The 4 congeners with detectable levels were 1,2,3,4,6,7,8-HpCDF, OCDF, 1,2,3,4,6,7,8-HpCDD, and OCDD, with reported detected levels of 0.0484, 0.0732, 0.246, and 0.738 ng/kg, respectively. The laboratory report includes calculated estimates of the total amount of dioxin and dioxin like compounds in the berberine product, expressed as total equivalents (or TEQ), derived using the WHO 2005 Total Equivalency Factors (TEF). Since the majority of the congeners is below the limit of reporting, the lab calculated 3 estimates of the total TEQ:

- A "lower bound" estimate that assumes that the actual levels for those congeners that were below the limit of reporting are zero

[1] total equivalents (or TEQ)

- An "upper bound" estimate that assumes that the actual levels for those congeners that were below the limit of reporting are equal to the limit of reporting
- A "medium bound" estimate that assumes that the actual levels for those congeners that were below the limit of reporting are equal to ½ the limit of reporting.

Table 1. Dioxin levels in the berberine product, as reported by the lab

Congener	Concentration (ng/kg)	WHO-TEF (2005)	WHO-TEQ (ng/kg)[a]		
			Lower bound[b]	Medium bound[c]	Upper bound[d]
2,3,7,8-TCDF	<0.0213	0.1	0	0.00107	0.00213
1,2,3,7,8-PeCDF	<0.0126	0.03	0	0.000189	0.000379
2,3,4,7,8-PeCDF	<0.0128	0.3	0	0.00191	0.00383
1,2,3,4,7,8-HxCDF	<0.00923	0.1	0	0.000462	0.000923
1,2,3,6,7,8-HxCDF	<0.00890	0.1	0	0.000445	0.00089
2,3,4,6,7,8-HxCDF	<0.00850	0.1	0	0.000425	0.00085
1,2,3,7,8,9-HxCDF	<0.0117	0.1	0	0.000584	0.00117
1,2,3,4,6,7,8-HpCDF	0.0484	0.01	0.000484	0.000484	0.000484
1,2,3,4,7,8,9-HpCDF	<0.0131	0.01	0	0.0000654	0.000131
OCDF	0.0732	0.0003	0.000022	0.000022	0.000022
2,3,7,8-TCDD	<0.0182	1	0	0.0091	0.0182
1,2,3,7,8-PeCDD	<0.0211	1	0	0.0106	0.0211
1,2,3,4,7,8-HxCDD	<0.00731	0.1	0	0.000365	0.000731
1,2,3,6,7,8-HxCDD	<0.00731	0.1	0	0.000365	0.000731
1,2,3,7,8,9-HxCDD	<0.00770	0.1	0	0.000385	0.00077
1,2,3,4,6,7,8-HpCDD	0.246	0.01	0.00246	0.00246	0.00246
OCDD	0.738	0.0003	0.000222	0.000222	0.000222
Total TEQ			0.00319	0.0291	0.055

[a] Derived by multiplying the congener specific concentration by its corresponding 2005-WHO-TEF
[b] Derived assuming a zero concentration for congeners with levels as below the limit of reporting
[c] Derived assuming a concentration equal to ½ the limit of reporting for congeners with levels reported as below the limit of reporting
[d] Derived assuming a concentration equal to the limit of reporting for congeners with levels reported as below the limit of reporting

B. Comparison of levels in the berberine product with levels reported by CVM in poultry feed samples

Together with the US Environmental Protection Agency (EPA), CVM initiated a survey in 2000 where 47 feed samples were collected and analyzed for DLC. The results of the analysis of 44 of these samples were reported by Ferrario el al (2002)[2], and summarized in a 2003 Institute of Medicine (IOM) report[3].

[2] Ferrario, J., R. Lovell, P. Gardner, M. Lorber, D. Winters, AND C. Byrne. Analysis Of Animal-And Plant-Derived Feed Ingredients For Dioxin-Like Compounds. ORGANOHALOGEN COMPOUNDS 57:85-88, (2002).
[3] Institute of Medicine 2003. Dioxins and Dioxin-like Compounds in the Food Supply: Strategies to Decrease Exposure. Washington, DC: The National Academies Press. https://doi.org/10.17226/10763.

Of the 44 feed samples, 29 were animal-based ingredients and the remaining 19 were plant based, namely deodorizer distillates and molasses, corn oil, and canola meal. Table 2 summarizes the results in the plant-based feed ingredients in the CVM survey, as reported by IOM. The average 1998-WHO total TEQ levels in the deodorizer distillate samples was 3.35 ng/kg and 3.38 ng/kg, when non-detect (ND) levels were assumed to be zero or ½ the limit of detection (LOD), respectively. For the molasses, corn oil, and canola meal samples, the average 1998-WHO total TEQ levels were 0.02 ng/kg (ND=zero) and 0.13 ng/kg (ND=½LOD).

The 0.003 ng/kg (lower bound) and 0.029 ng/kg (medium bound) total 1998-WHO TEQ levels in the berberine product are lower than the 0.02 ng/kg and 3.35 ng/kg (ND=0) and 0.13 ng/kg and 3.38 ng/kg (ND=½ LOD) total 1998-TEQ levels reported in the deodorizer distillate and molasses, corn oil, and canola meal samples. The 4 congeners that were above the reporting limit in the berberine product (1,2,3,4,6,7,8-HpCDF, OCDF, 1,2,3,4,6,7,8-HpCDD, and OCDD) are also the congeners that have the highest reported mean values for plant based animal feed in the FDA CVM survey, and the levels in the berberine product are well below the mean levels reported in the CVM samples for these 4 congeners (see Table 2).

Table 2. Comparison of the dioxin levels in the berberine product and the FDA CVM plant based feed samples

	Concentration (ng/kg)			
		FDA CVM Plant-Based Feed		
		Deodorizer distillates	**Molasses, corn oil, and canola meal**	1998-WHO
Congener	Lab Sample	(n=11)	(n=8)	TEF
2,3,7,8-TCDF	<0.0213	0.68	0.02	0.1
1,2,3,7,8-PeCDF	<0.0126	0.4	0.04	0.05
2,3,4,7,8-PeCDF	<0.0128	0.77	0.04	0.5
1,2,3,4,7,8-HxCDF	<0.00923	0.7	0.04	0.1
1,2,3,6,7,8-HxCDF	<0.00890	0.51	0.04	0.1
2,3,4,6,7,8-HxCDF	<0.00850	0.65	0.04	0.1
1,2,3,7,8,9-HxCDF	<0.0117	0.24	0.05	0.1
1,2,3,4,6,7,8-HpCDF	**0.0484**	**6.54**	**0.5**	**0.01**
1,2,3,4,7,8,9-HpCDF	<0.0131	0.57	0.12	0.01
OCDF	**0.0732**	**10.55**	**1.63**	**0.0001**
2,3,7,8-TCDD	<0.0182	0.23	0.02	1
1,2,3,7,8-PeCDD	<0.0211	0.92	0.04	1
1,2,3,4,7,8-HxCDD	<0.00731	1.31	0.04	0.1
1,2,3,6,7,8-HxCDD	<0.00731	3.27	0.04	0.1
1,2,3,7,8,9-HxCDD	<0.00770	3.45	0.04	0.1
1,2,3,4,6,7,8-HpCDD	**0.246**	**62.55**	**1.12**	**0.01**
OCDD	**0.738**	**511.15**	**11.61**	**0.0001**
Total 1998-WHO TEQ				
Assuming ND=0	0.003	3.35	0.02	
Assuming ND=1/2 LOD	0.030	3.38	0.13	

C. Comparison to levels detected in poultry samples collected by FDA and FSIS

1. Estimation of potential level in poultry meat products

The potential concentrations of the various DLC congeners in chicken meat were derived using the following standard formulas[4]:

$A_{poultry} = C_{fat} \times 0.1$

$$C_{fat} = BCF_{poultry} x (DF_{poultry_{soil}} x B_s x C_{soil} + DF_{poultry_{forage}} x P_{forage} + DF_{poultry_{feed}} x P_{feed}) \quad (1)$$

Where:

$A_{poultry}$ is the concentration of DLC in chicken poultry (mg/kg);

0.1 is the fraction of fat in poultry (unitless);

C_{fat} is the concentration of the specific congener in chicken fat (mg/kg);

$BCF_{poultry}$ is the congener-specific bioconcentration ratio of the congener developed for chicken vegetative intakes (unitless)[5] (see Table 3);

$DF_{poultry_{soil}}$ and $DF_{poultry_{forage}}$ are the fractions of chicken diets that are soil and incidental vegetation while free ranging, and assumed, conservatively, to be zero in the current assessment (unitless);

B_s, C_{soil}, and P_{forage} are the bioavailability of the contaminant on the soil vehicle relative to the vegetative vehicle (unitless), the contaminant concentration in the soil (mg/kg), and the contaminant concentration on free-range vegetation (mg/kg);

$DF_{poultry_{feed}}$ is the fraction of chicken diets that is feed, and assumed in the current assessment, conservatively, to be 100% in the current assessment (unitless);

P_{feed} is the contaminant concentration in feed (mg/kg).

Assuming conservatively that the entire chicken diet consists of the berberine product, formula (1) above reduces to: $C_{fat} = BCF_{poultry} x\ 100\% \times P_{feed}$.

Table 3. Poultry BCF

CAS	BCF	Chemical name	Table in RTI report
35822-46-9	1.4	1,2,3,4,6,7,8-HpCDD	D-3
67562-39-4	1	1,2,3,4,6,7,8-HpCDF	D-4
55673-89-7	0.9	1,2,3,4,7,8,9-HpCDF	D-5
39227-28-6	3.6	1,2,3,4,7,8-HxCDD	D-9

[4] Center for Environmental Analysis, RTI, Exposure Analysis of Dioxins, Dibenzofurans, and Coplanar Polychlorinated Biphenyls in Sewage Sludge (2003). (Formula 5-16, page 5-40)

[5] Provided in Appendix D in Center for Environmental Analysis, RTI, Exposure Analysis of Dioxins, Dibenzofurans, and Coplanar Polychlorinated Biphenyls in Sewage Sludge (2003).

CAS	BCF	Chemical name	Table in RTI report
57653-85-7	5.6	1,2,3,6,7,8-HxCDD	D-10
19408-74-3	2.4	1,2,3,7,8,9-HxCDD	D-11
70648-26-9	4.8	1,2,3,4,7,8-HxCDF	D-12
57117-44-9	5.3	1,2,3,6,7,8-HxCDF	D-13
72918-21-9	4.1	1,2,3,7,8,9-HxCDF	D-14
60851-34-5	2.1	2,3,4,6,7,8-HxCDF	D-15
3268-87-9	0.3	1,2,3,4,6,7,8,9-OCDD	D-16
39001-02-0	0.3	1,2,3,4,6,7,8,9-OCDF	D-17
40321-76-4	6.8	1,2,3,7,8-PeCDD	D-23
57117-41-6	18	1,2,3,7,8-PeCDF	D-24
57117-31-4	7.4	2,3,4,7,8-PeCDF	D-25
1746-01-6	8.8	2,3,7,8-TCDD	D-26
51207-31-9	3.1	2,3,7,8-TCDF	D-27
Maximum BCF	18		

Two estimates of the potential concentration in chicken fat were calculated assuming the entire chicken diet consist of the berberine product. The first used the congener specific BCF, while the other conservatively applied the maximum BCF to the total TEQ concentration in the berberine product. Both estimates assume that the dioxin level for the congeners reported as below the limit of reporting is equal to ½ the limit of reporting (see table 4). The estimated concentrations in chicken were derived assuming 10% fat in chicken.

Table 4. Calculated dioxin concentrations in chicken fat and meat associated with the berberine product[a]

	1998-WHO TEQ	2005-WHO TEQ
Using congener specific BCF		
Concentration in chicken fat (ng/kg)[b]	0.2006	0.1889
Concentration in chicken (ng/kg)[b,c]	0.0201	0.0189
Using maximum BCF		
Concentration in chicken fat (ng/kg)[b]	0.5464	0.5240
Concentration in chicken (ng/kg)[b,c]	0.0546	0.0524

[a] Assumes the entire chicken diet consists of the berberine product (see Table 3)
[b] Assumes that the dioxin level for the congeners reported as below the limit of reporting is equal to ½ the limit of reporting
[c] Assumes 10% fat in chicken (see Table 3)

2. <u>FDA chicken samples</u>

The US FDA has been monitoring food and animal feed with the goal of identifying ways to reduce dietary exposure to ubiquitous environmental contaminants. As part of FDA's Dioxin Monitoring Program, FDA analyzes foods collected under its Total Diet Study (TDS) as well as additional non-TDS foods. Data on dioxin levels in chicken are only available in the TDS. The TDS is an ongoing FDA program that collects samples of approximately 280 foods and beverages that are representative of the average U.S. diet and analyzes these samples for about 800 contaminants and nutrients. While the TDS

samples are typically collected from four representative areas of the country, four times per year (once per region), only one sample per food/beverage per year was analyzed for dioxin. Dioxin data from the TDS are available for 2000-2004, however, the 2000 results are only available in summary form, hence only data from the 2001-2004 dioxin TDS sampling are used in the current assessment.

Twenty-two (22) of the foods sampled in the 2001-2004 TDS were identified as "chicken foods". Dioxin levels reported in FDA's TDS use the 1998-WHO TEF. The minimum and maximum total 1998-WHO total TEQ levels for the TDS chicken foods are summarized in Table 5.

Table 5. Summary of reported total 1998-WHO TEQ levels in chicken foods identified in the FDA TDS database

	1998-WHO TEQ Levels (ng/kg)					
	ND assumed at zero		ND assumed at ½ LOD		ND assumed at LOD	
FDA TDS food	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
BF, chicken and broth/gravy	0	0	0.0056	0.0063	0.0112	0.0126
BF, chicken noodle dinner	0.0085	0.0321	0.0409	0.0476	0.0631	0.0732
BF, chicken w/ rice	0.0019	0.0134	0.0124	0.0141	0.0148	0.0229
BF, vegetables and chicken	0	0	0.0032	0.0128	0.0064	0.0255
Chicken breast, fried, fast-food (w/ skin)	0	0.0025	0.0079	0.0132	0.0158	0.024
Chicken breast, oven-roasted (skin removed)	0.0065	0.018	0.0109	0.0255	0.0153	0.0329
chicken breast, roasted	0.0083	0.0218	0.0166	0.0326	0.0248	0.0433
Chicken filet (broiled) sandwich on bun, fast-food	0	0	0.012	0.0142	0.024	0.0284
Chicken leg, fried, fast-food (w/ skin)	0	0.0014	0.0058	0.0106	0.0117	0.0198
chicken noodle dinner, st/jr	0	0.0019	0.0068	0.0115	0.0131	0.021
chicken noodle soup, canned	0.0001	0.0013	0.0024	0.009	0.0036	0.0179
Chicken nuggets, fast-food	0	0.0052	0.0077	0.0164	0.0153	0.0317
chicken potpie, frozen	0.01	0.0475	0.0196	0.0868	0.0292	0.1285
Chicken potpie, frozen, heated	0.0071	0.0214	0.016	0.028	0.0248	0.0346
Chicken thigh, oven-roasted (skin removed)	0	0.0015	0.0084	0.0191	0.0168	0.0366
Chicken w/ vegetables in sauce, from Chinese carry-out	0	0.0069	0.0117	0.0175	0.0234	0.0281
chicken, fried homemade	0.0312	0.0556	0.0559	0.0636	0.0562	0.0961
chicken, fried, fast-food	0.0017	0.0392	0.0306	0.058	0.0595	0.0768
chicken, st/jr	0	0.0008	0.0094	0.0343	0.018	0.0686
Luncheon meat (chicken/turkey)	0	0.0016	0.0106	0.0128	0.0213	0.024
Soup, chicken noodle, canned, cond, prep w/ water	0	0	0.0017	0.0086	0.0034	0.0172
vegetables and chicken, st/jr	0	0.0001	0.0024	0.0816	0.0046	0.1631
All samples	0	0.0556	0.0017	0.0868	0.0034	0.1631

3. USDA FSIS Chicken samples

The USDA conducted surveys of US domestic meat and poultry samples in 2002–2003, 2007–2008, and in 2012-2013. The total TEQ levels, expressed as 2005-WHO TEQ for 153 chicken samples collected in

2012-2013, the most recent USDA FSIS survey[6] are used in the current assessment. The data are summarized in Table 6.

Table 6. Summary TEQ Values for Young Chicken Samples (n=153) in the USDA FSIS Dioxin 2012-2013 Survey

	2005-WHO total TEQ (ng TEQ/kg lipid)	
	ND=0	ND=LOD/2
Mean	0.060	0.114
Min	0.001	0.024
Max	1.822	1.835

4. Comparison of the estimated dioxin levels in chicken associated with the berberine product and the FDA TDS and USDA FSIS levels

Table 6 compares the calculated 1998-WHO total TEQ dioxin levels in chicken associated with the berberine product to the range of levels reported for chicken food samples in the FDA-TDS database and for young chicken in USDA FSIS database. The calculated 2005-WHO total TEQ dioxin level in chicken fat are within the ranges detected in the FDA-TDS ad USDA FSIS databases.

	Calculated level associated with the berberine product		**Range of levels**	
	Using congener specific BCF	**Using Maximum BCF**	**FDA TDA chicken samples**	**USDA FSIS chicken samples**
1998-WHO Total TEQ levels (ng/kg)	0.0201 (chicken)	0.0546 (chicken fat)	0.0017-0.0868	-
2005-WHO Total TEQ levels (ng/kg)	0.1889 (chicken fat)	0.5240 (chicken fat)	-	0.024 – 1.835

[6] See Table 9 in USDA FSIS, USDA, DIOXIN FY2013 Survey: Dioxins and Dioxin-Like Compounds in the U.S. Domestic Meat and Poultry Supply (2015).

Attachment A. Lab Report

(b) (4)

Test Report

REPORT No.: (b) (4)

ATTENTION: -

DATE RECEIVED: 2020/03/17

CUSTOMER: (b)(6)

DATE REPORTED: 2020/03/23

ADDRESS:

SAMPLE (S): Berberine Hydrochloride (1)

REMARKS

1.The results apply to the sample(s) as received.

2.The report is translated from (b) (4)

Edited by (b)(6)

Reviewed by

Approved by

(b) (4) Authorized Signatory

(b) (4)

声明

Statement

1. FOREIGN LANGUAGE

The test report is invalid without the official seal of the laboratory.

2. FOREIGN LANGUAGE

This test report cannot be reproduced in any way, except in full content, without prior approval in writing by the laboratory.

3. FOREIGN LANGUAGE

The test report is invalid without the signature of the compiler, the checker and the approver

4. FOREIGN LANGUAGE

The test report is invalid if altered.

5. FOREIGN LANGUAGE

The test report has been drafted in Chinese and translated into English (if applicable) for convenience only. In the event of discrepancy, the Chinese version shall prevail.

符号表/Legend

NA FOREIGN LANGUAGE he sample was not analysed for this analyte

↑ FOREIGN LANGUAGE Detection limit raised

↓ Detection limit lowered

ND FOREIGN LANGUAGE Not Detected

(b) (4)

DIOXIN ANALYSIS

Report No.:SHE20-01793/1

Customer Reference:-

Lab ID	(b) (4)
Customer ID	Berberine Hydrochloride
Sample Batch	CHL-C-A009051
Date Received	2020/03/17

ITEM	METHOD	Concen-tration (ng/kg)	WHO-TEF (2005)	WHO-TEQ (ng/kg) Lowerbound	WHO-TEQ (ng/kg) Mediumbound	WHO-TEQ (ng/kg) Upperbound	WHO-TEQ (ng/kg) Limit of Reporting
PCDDs & PCDFs							
2,3,7,8-TCDF	GB 5009.205-2013	< 0.0213	0.1	0	0.00107	0.00213	0.00213
1,2,3,7,8-PeCDF		< 0.0126	0.03	0	0.000189	0.000379	0.000379
2,3,4,7,8-PeCDF		< 0.0128	0.3	0	0.00191	0.00383	0.00383
1,2,3,4,7,8-HxCDF		< 0.00923	0.1	0	0.000462	0.000923	0.000923
1,2,3,6,7,8-HxCDF		< 0.00890	0.1	0	0.000445	0.000890	0.000890
2,3,4,6,7,8-HxCDF		< 0.00850	0.1	0	0.000425	0.000850	0.000850
1,2,3,7,8,9-HxCDF		< 0.0117	0.1	0	0.000584	0.00117	0.00117
1,2,3,4,6,7,8-HpCDF		**0.0484**	0.01	0.000484	0.000484	0.000484	0.0000677
1,2,3,4,7,8,9-HpCDF		< 0.0131	0.01	0	0.0000654	0.000131	0.000131
OCDF		**0.0732**	0.0003	0.0000220	0.0000220	0.0000220	0.00000556
2,3,7,8-TCDD		< 0.0182	1	0	0.00910	0.0182	0.0182
1,2,3,7,8-PeCDD		< 0.0211	1	0	0.0106	0.0211	0.0211
1,2,3,4,7,8-HxCDD		< 0.00731	0.1	0	0.000365	0.000731	0.000731
1,2,3,6,7,8-HxCDD		< 0.00731	0.1	0	0.000365	0.000731	0.000731
1,2,3,7,8,9-HxCDD		< 0.00770	0.1	0	0.000385	0.000770	0.000770
1,2,3,4,6,7,8-HpCDD		**0.246**	0.01	0.00246	0.00246	0.00246	0.0000970
OCDD		**0.738**	0.0003	0.000222	0.000222	0.000222	0.00000486
Total(PCDDs & PCDFs)				0.00319	0.0291	**0.0550**	0.0520

Remark:

WHO-TEF: Toxic Equivalency Factor

Lowerbound concentration : For the calculation of the total concentration.
the values. which were lower than the limit of reporting. were regarded as zero.

Mediumbound concentration : For the calculation of the total mediumbound concentration.
the values.which were lower than the limit of reporting. were regarded as the half of the limit of reporting.

Upperbound concentration :For the calculation of the total upperbound concentration.
the values. which were lower than the limit of reporting. were regarded as the value of the limit of reporting.

(b) (4)

DIOXIN QC

Report No.:SHE20-01793/1

Customer Reference:-

Lab ID	(b) (4)
Customer ID	Berberine Hydrochloride
Sample Batch	CHL-C-A009051
Date Received	2020/03/17

ITEM	METHOD	Extraction Standards Rec(%)
Extraction Standards Rec(%)		
^{13}C-2,3,7,8-TCDF	GB 5009.205-2013	(b) (4)
^{13}C-1,2,3,7,8-PeCDF		
^{13}C-2,3,4,7,8-PeCDF		
^{13}C-1,2,3,4,7,8-HxCDF		
^{13}C-1,2,3,6,7,8-HxCDF		
^{13}C-2,3,4,6,7,8-HxCDF		
^{13}C-1,2,3,7,8,9-HxCDF		
^{13}C-1,2,3,4,6,7,8-HpCDF		
^{13}C-1,2,3,4,7,8,9-HpCDF		
^{13}C-2,3,7,8-TCDD		
^{13}C-1,2,3,7,8-PeCDD		
^{13}C-1,2,3,4,7,8-HxCDD		
^{13}C-1,2,3,6,7,8-HxCDD		
^{13}C-1,2,3,4,6,7,8-HpCDD		
^{13}C-OCDD		
Purify Standard Rec(%)		
$^{37}Cl_4$-2,3,7,8-TCDD	GB 5009.205-2013	

Remark:

1):Sample information is from the customer.

End of Report

Appendix E. PCBs Analytical Results

(b) (4)

Test Report

(b) (4)

Date: 31 Jul 2020

Client Name:
Client Address: (b) (4)

Sample Name:	Berberine Hydrochloridet
Manufacturer:	/
Sample Batch No.:	CYSXBJ-C-A009920
Production Date:	/

Above information and sample(s) was/were submitted and certified by the client, (b) (4) quoted the information with no responsibility as to the accuracy, adequacy and/or completeness.

Date of Sample Received :	26 Jul 2020
Testing Period :	26 Jul 2020 - 31 Jul 2020
Test Requested :	Selected test(s) as requested by client.
Test Method :	Please refer to next page(s).
Test Result(s) :	Please refer to next page(s).

Chinese shall prevail in this report.

Unless otherwise stated the results shown in this test report refer only to the items tested. This document cannot be used for publicity, without prior written approval of the (b) (4)

(b) (4)

(b) (4)

PREPARED BY CHECKED BY APPROVED BY

(b) (4)

(b) (4)

(b) (4)

Test Report (b) (4) Date: 31 Jul 2020

Sample Description :

Specimen No.	(b) (4) Sample ID	Description
1	(b) (4)	solid in bag

Chemical test

Test Result(s) :

Test Item(s)	Unit	Test Method(s)	Test Result(s) 001	LOQ
2.4.4'-Trichlorobiphenyl (PCB 28)	µg/kg	GB 5009.190-2014 II	ND	5
2.2'.5.5'-Tetrachloro-biphenyl (PCB 52)	µg/kg	GB 5009.190-2014 II	ND	5
2.2'.4.5.5'-Pentachloro-biphenyl (PCB 101)	µg/kg	GB 5009.190-2014 II	ND	5
2.3'.4.4'.5-Pentachlorobiphenyl (PCB 118)	µg/kg	GB 5009.190-2014 II	ND	5
2.2'3.4.4'.5'-Hexachloro-biphenyl (PCB 138)	µg/kg	GB 5009.190-2014 II	ND	5
2.2'.4.4'.5.5'-Hexachloro-biphenyl (PCB 153)	µg/kg	GB 5009.190-2014 II	ND	5
2.2'.3.4.4'.5.5'-Heptachlorobiphenyl (PCB 180)	µg/kg	GB 5009.190-2014 II	ND	5
PCBs (Polychlorinated Biphenyls) content	µg/kg	GB 5009.190-2014 II	ND	-

(b) (4)

Remark:
1.ND = Not Detected
2.LOQ = Limit of Quantitation

*** End ***

(b) (4)

(b) (4)

Appendix F. Stability Study Reports

Stability in mash and pellets for broiler chickens

(b) (4)

Concentration and Stability of Additives in Poultry Feed

(b) (4) A52359

(b) (4)

(b) (4)

CONFIDENTIAL REPORT

Prepared by

(b) (4)

October 19, 2018

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Project Objective

Determine the concentrations of berberine, (b) (4) in chicken feed at the initial sample delivery time (T0) and one week (T0 + 7) and two weeks (T0 + 14) later (samples were shipped on ice but stored at room temperature after the T0 samples were taken).

Samples Tested

Sample Description	(b) (4)	Date Received	Date(s) Extracted
(b) (4)	(b) (4)	6/7/2018	Method dev and standards
(b) (4)	(b) (4)	6/7/2018	Method dev and standards
Berberine Hydrochloride (97%)	(b) (4)	6/7/2018	Method dev and standards
Berberine: 0.03g/kg feed		9/27/18	9/28, 10/5, 10/12
(b) (4)		9/27/18	9/28, 10/5, 10/12
(b) (4)		9/27/18	9/28, 10/5, 10/12
(b) (4)		9/27/18	9/28, 10/5, 10/12
(b) (4)		9/27/18	9/28, 10/5, 10/12
(b) (4) (b) (4)		9/27/18	9/28, 10/5, 10/12
(b) (4) (b) (4)		9/27/18	9/28, 10/5, 10/12

The results presented in this report apply only to the samples tested.
Samples were received in good condition in a cooler on ice and contained both pellet and mash.

Methodology

(b) (4)

(b) (4)

All standards and samples were injected into a LCMS using the conditions in Table 1.

Table 1. LCMS Conditions

HPLC	
LC Model	(b) (4)
LC Column	
LC Flow Rate	
LC Injection Volume	
LC Column Temperature	
LC Eluent A	
LC Eluent B	

LC Gradient		
Time (minutes)	Eluent A (%)	Eluent B (%)
(b) (4)		(b) (4)
Run Time	(b) (4)	
Post Time		

Mass Spectrometer	
MS Model	(b) (4)
ESI (electrospray ionization)	
ESI Source	
TOF (Time of Flight) Range	
Ions of Interest	

(b) (4)

Table 2. Spike Recoveries (%) of Berberine

(b) (4)

(b) (4)

Table 3. Spike Recoveries (%) of (b) (4)

(b) (4)

Table 4. Spike Recoveries (%) of (b) (4)

(b) (4)

Table 5. Sequential Extraction Results

(b) (4)

Figure 1. Calibration Curves used to Quantitate Sample Batch 1

(b) (4)

(b) (4)

Results

(b) (4)

Table 6. (b) (4)

(b) (4)

(b) (4)

Table 7. Results for Berberine

(b) (4)

(b) (4)

Table 8. (b) (4)

(b) (4)

Reviewers

Prepared by: (b)(6) Date: October 19, 2018

Reviewed by: Date: October 19, 2018

Reviewed by: Date: October 19, 2018

Appendix G. Efficacy Study Report

Appendix H. FNDDS Ingredients with Chicken-Derived Components Used in the Analysis of EDIs

FNDDS Ingredients with Chicken-Derived Components Used in the Analysis of EDIs

Ingredient Code	Ingredient Description
3012	Babyfood, meat, chicken, strained
3013	Babyfood, meat, chicken, junior
3014	Babyfood, meat, chicken sticks, junior
3068	Babyfood, dinner, chicken noodle, strained*
3069	Babyfood, dinner, chicken noodle, junior*
3070	Babyfood, dinner, chicken soup, strained*
3072	Babyfood, dinner, chicken stew, toddler*
3073	Babyfood, dinner, vegetables chicken, strained*
3274	Babyfood, dinner, vegetables and chicken, junior*
3297	Babyfood, dinner, apples and chicken, strained*
3298	Babyfood, dinner, broccoli and chicken, junior*
3303	Babyfood, dinner, sweet potatoes and chicken, strained*
4542	Fat, chicken*
5000	Chicken, broiler, rotisserie, BBQ, breast meat only
5004	Chicken, broilers or fryers, meat and skin and giblets and neck, roasted
5006	Chicken, broilers or fryers, meat and skin, raw
5007	Chicken, broilers or fryers, meat and skin, cooked, fried, batter*
5008	Chicken, broilers or fryers, meat and skin, cooked, fried, flour*
5009	Chicken, broilers or fryers, meat and skin, cooked, roasted
5010	Chicken, broilers or fryers, meat and skin, cooked, stewed
5013	Chicken, broilers or fryers, meat only, roasted
5014	Chicken, broilers or fryers, meat only, stewed
5015	Chicken, broilers or fryers, skin only, raw
5018	Chicken, broilers or fryers, skin only, cooked, roasted
5022	Chicken, broilers or fryers, giblets, cooked, simmered
5024	Chicken, gizzard, all classes, cooked, simmered
5027	Chicken, liver, all classes, raw
5028	Chicken, liver, all classes, cooked, simmered
5041	Chicken, broilers or fryers, light meat, meat only, cooked, roasted
5042	Chicken, broilers or fryers, light meat, meat only, cooked, stewed
5045	Chicken, broilers or fryers, dark meat, meat only, cooked, roasted
5046	Chicken, broilers or fryers, dark meat, meat only, cooked, stewed
5047	Chicken, broilers or fryers, separable fat, raw
5051	Chicken, broilers or fryers, back, meat and skin, cooked, roasted
5052	Chicken, broilers or fryers, back, meat and skin, cooked, stewed
5057	Chicken, broilers or fryers, breast, meat and skin, raw
5060	Chicken, broilers or fryers, breast, meat and skin, cooked, roasted
5061	Chicken, broilers or fryers, breast, meat and skin, cooked, stewed
5062	Chicken, broiler or fryers, breast, skinless, boneless, meat only, raw
5063	Chicken, broilers or fryers, breast, meat only, cooked, fried
5064	Chicken, broilers or fryers, breast, meat only, cooked, roasted

5065	Chicken, broilers or fryers, breast, meat only, cooked, stewed
5066	Chicken, broilers or fryers, drumstick, meat and skin, raw
5069	Chicken, broilers or fryers, drumstick, meat and skin, cooked, roasted
5070	Chicken, broilers or fryers, drumstick, meat and skin, cooked, stewed
5071	Chicken, broilers or fryers, dark meat, drumstick, meat only, raw
5073	Chicken, broilers or fryers, dark meat, drumstick, meat only, cooked, roasted
5074	Chicken, broilers or fryers, drumstick, meat only, cooked, stewed
5087	Chicken, broilers or fryers, neck, meat and skin, cooked simmered
5091	Chicken, broilers or fryers, thigh, meat and skin, raw
5094	Chicken, broilers or fryers, thigh, meat and skin, cooked, roasted
5095	Chicken, broilers or fryers, thigh, meat and skin, cooked, stewed
5096	Chicken, broilers or fryers, dark meat, thigh, meat only, raw
5098	Chicken, broilers or fryers, thigh, meat only, cooked, roasted
5099	Chicken, broilers or fryers, thigh, meat only, cooked, stewed
5100	Chicken, broilers or fryers, wing, meat and skin, raw
5103	Chicken, broilers or fryers, wing, meat and skin, cooked, roasted
5104	Chicken, broilers or fryers, wing, meat and skin, cooked, stewed
5105	Chicken, broilers or fryers, wing, meat only, raw
5107	Chicken, broilers or fryers, wing, meat only, cooked, roasted
5108	Chicken, broilers or fryers, wing, meat only, cooked, stewed
5308	Chicken, cornish game hens, meat and skin, cooked, roasted
5310	Chicken, cornish game hens, meat only, cooked, roasted
5311	Chicken, canned, no broth
5314	Chicken, broilers or fryers, breast, skinless, boneless, meat only, with added solution, raw
5319	Chicken, broiler, rotisserie, BBQ, drumstick, meat only
5324	Chicken patty, frozen, cooked*
5334	Chicken, broiler, rotisserie, BBQ, thigh, meat only
5335	Chicken, feet, boiled
5342	Chicken, broilers or fryers, breast, meat only, cooked, rotisserie, original seasoning
5358	Chicken, broiler, rotisserie, BBQ, breast meat and skin
5359	Chicken, broiler, rotisserie, BBQ, drumstick meat and skin
5361	Chicken, broiler, rotisserie, BBQ, thigh meat and skin
5362	Chicken, broiler, rotisserie, BBQ, wing meat and skin
5671	Chicken, broilers or fryers, dark meat, drumstick, meat only, cooked, braised
5672	Chicken, broilers or fryers, dark meat, thigh, meat only, cooked, braised
5676	Chicken, broilers or fryers, dark meat, drumstick, meat and skin, cooked, braised
5677	Chicken, broilers or fryers, dark meat, thigh, meat and skin, cooked, braised
5678	Chicken, dark meat, drumstick, meat only, with added solution, raw
5679	Chicken, dark meat, drumstick, meat only, with added solution, cooked, roasted
5680	Chicken, dark meat, drumstick, meat only, with added solution, cooked, braised
5681	Chicken, dark meat, thigh, meat only, with added solution, cooked, braised
5682	Chicken, dark meat, thigh, meat only, with added solution, raw
5683	Chicken, dark meat, thigh, meat only, with added solution, cooked, roasted
5687	Chicken, dark meat, drumstick, meat and skin, with added solution, cooked, braised
5688	Chicken, dark meat, drumstick, meat and skin, with added solution, raw
5689	Chicken, dark meat, drumstick, meat and skin, with added solution, cooked, roasted
5690	Chicken, dark meat, thigh, meat and skin, with added solution, cooked, braised
5691	Chicken, dark meat, thigh, meat and skin, with added solution, raw

5692	Chicken, dark meat, thigh, meat and skin, with added solution, cooked, roasted
6015	Soup, chicken, canned, chunky, ready-to-serve*
6016	Soup, cream of chicken, canned, condensed*
6018	Soup, chunky chicken noodle, canned, ready-to-serve*
6019	Soup, chicken noodle, canned, condensed*
6022	Soup, chicken rice, canned, chunky, ready-to-serve*
6023	Soup, chicken with rice, canned, condensed*
6024	Soup, chicken and vegetable, canned, ready-to-serve*
6029	CAMPBELL'S, HEALTHY REQUEST, chicken with rice, condensed*
6119	Gravy, chicken, canned or bottled, ready-to-serve*
6128	Soup, chicken noodle, dry, mix*
6149	Soup, chicken mushroom, canned, condensed*
6208	Soup, chicken vegetable with potato and cheese, chunky, ready-to-serve*
6417	Soup, chicken gumbo, canned, prepared with equal volume water*
6955	Soup, cream of chicken, canned, condensed, reduced sodium*
6977	Gravy, meat or poultry, low sodium, prepared*
7021	Dutch brand loaf, chicken, pork and beef*
7024	Frankfurter, chicken
7053	Pate, chicken liver, canned*
7067	Poultry salad sandwich spread*
7083	Sausage, Vienna, canned, chicken, beef, pork*
7906	Luncheon meat, pork, ham, and chicken, minced, canned, reduced sodium, added ascorbic acid, includes SPAM, 25% less sodium*
7909	Luncheon meat, pork and chicken, minced, canned, includes SPAM Lite*
7961	Chicken breast, deli, rotisserie seasoned, sliced, prepackaged
7962	Frankfurter, meat and poultry, unheated*
7971	Bologna, meat and poultry*
7976	Sausage, chicken or turkey, Italian style, lower sodium
16008	Beans, baked, canned, with franks*
21010	Fast foods, biscuit, with crispy chicken fillet*
21102	Fast foods, chicken fillet sandwich, plain with pickles*
21124	Fast foods, submarine sandwich, cold cut on white bread with lettuce and tomato*
21151	Fast foods, submarine sandwich, sweet onion chicken teriyaki on white bread with lettuce, tomato and sweet onion sauce*
21154	Fast foods, submarine sandwich, oven roasted chicken on white bread with lettuce and tomato*
21229	Fast foods, chicken, breaded and fried, boneless pieces, plain*
21230	Fast Foods, crispy chicken filet sandwich, with lettuce and mayonnaise*
21401	Fast foods, chicken tenders*
21461	Fast foods, grilled chicken, bacon and tomato club sandwich, with cheese, lettuce, and mayonnaise*
21462	Fast foods, crispy chicken, bacon, and tomato club sandwich, with cheese, lettuce, and mayonnaise*
21464	Fast Foods, Fried Chicken, Breast, meat only, skin and breading removed
21465	Fast Foods, Fried Chicken, Drumstick, meat only, skin and breading removed
21466	Fast Foods, Fried Chicken, Thigh, meat only, skin and breading removed
21467	Fast Foods, Fried Chicken, Wing, meat only, skin and breading removed
21469	Fast Foods, Fried Chicken, Breast, meat and skin and breading*
21470	Fast Foods, Fried Chicken, Drumstick, meat and skin with breading*

21471	Fast Foods, Fried Chicken, Thigh, meat and skin and breading*
21472	Fast Foods, Fried Chicken, Wing, meat and skin and breading*
21487	Fast foods, taco with chicken, lettuce and cheese, soft*
21488	Fast foods, quesadilla, with chicken*
21490	Fast Foods, grilled chicken filet sandwich, with lettuce, tomato and spread*
21517	Fast foods, crispy chicken in tortilla, with lettuce, cheese, and ranch sauce*
21518	Fast foods, grilled chicken in tortilla, with lettuce, cheese, and ranch sauce*
21610	School Lunch, chicken patty, whole grain breaded*
21611	School Lunch, chicken nuggets, whole grain breaded*
22522	Pasta with Sliced Franks in Tomato Sauce, canned entree*
22535	HOT POCKETS, CROISSANT POCKETS Chicken, Broccoli, and Cheddar Stuffed Sandwich, frozen*
22906	Chicken pot pie, frozen entree, prepared*
22954	Egg rolls, chicken, refrigerated, heated*
22958	Rice bowl with chicken, frozen entree, prepared (includes fried, teriyaki, and sweet and sour varieties)*
22973	Corn dogs, frozen, prepared*
22974	Chicken, nuggets, dark and white meat, precooked, frozen, not reheated*
22978	Chicken tenders, breaded, frozen, prepared*
27044	Soup, chicken noodle, reduced sodium, canned, ready-to-serve*
32006	Taquitos, frozen, chicken and cheese, oven-heated*
32026	Turnover, chicken- or turkey-, and vegetable-filled, reduced fat, frozen*
36618	Restaurant, Chinese, general tso's chicken*
36619	Restaurant, Chinese, kung pao chicken*
36621	Restaurant, Chinese, sweet and sour chicken*
36626	Restaurant, Chinese, chicken and vegetables*
36629	Restaurant, Chinese, orange chicken*
36633	Restaurant, Chinese, sesame chicken*
42280	Frankfurter, meat and poultry, low fat*
43008	Babyfood, dinner, chicken and rice*
43373	Babyfood, dinner, chicken and noodle with vegetables, toddler*
43507	Frankfurter, low sodium*
99995	Chicken, NS as to part, roasted or broiled
99996	Chicken, NS as to part, stewed
99997	Chicken, breast, roasted, broiled, or baked, skin not eaten
907961	REDUCED SODIUM: Chicken breast, deli, rotisserie seasoned, sliced, prepackaged
907971	REDUCED SODIUM: Bologna, meat and poultry*

* Only the proportion of the ingredient corresponding to chicken was included in the analysis.

Appendix I. Signed GRAS Panel Statement

Consensus Opinion Statement of the GRAS Panel Concerning the Proposed Use of Berberine as a Preservative Against Yeast and Mold in Feed for Broiler Chickens

Introduction

The undersigned, an independent panel of experts, qualified by their scientific training and national and international experience to evaluate the safety of human and animal foods and food ingredients (the "GRAS Panel"), was specially convened to evaluate the safety and "generally recognized as safe" (GRAS) status of the intended use of berberine, marketed by IRP Health PTY LTD, also known as IRP Health Inc. ("IRP Health"), intended for use as a preservative in feed for broiler chickens. Berberine will be added to feed at a concentration of 0.03 g/kg to preserve the feed from yeast and mold. For purposes of this review, "safe" or "safety" means that there is "a reasonable certainty in the minds of competent scientists that the substance is not harmful under the conditions of its intended use," as defined by the United States Food and Drug Administration (FDA) in 21 CFR § 570.3(i).

The safety of the intended use of berberine as a preservative in feed for broiler chickens was previously concluded to be GRAS (2018 GRAS Panel). On Feb 12, 2020, the supporting data and information was summarized for FDA Center for Veterinary Medicine officials who then recommended that certain additional information be added to the supporting dossier. For the current dossier, Exponent, Inc. ("Exponent") performed an update of the scientific literature, through December 2020, relating to the safety of berberine. Exponent summarized results of the literature search and prepared an updated safety dossier, "Documentation Supporting the Generally Recognized as Safe (GRAS) Status of the Use of Berberine in Feed for Broiler Chickens" [September 28, 2020] for consideration by the GRAS Panel. Pharmacokinetic data and potential risk of microbial resistance are included in the updated dossier. In addition, the ingredient specifications, including impurities, the efficacy data and the estimated daily intakes (EDIs) are updated in the current dossier.

The GRAS Panel consists of the following qualified experts: William Allaben, Ph.D., ATS (Adjunct Professor, College of Medicine, College of Public Health, University of Arkansas for Medical Sciences; Toxicology Advisors, LLC), William Dozier, Ph.D. (Professor, Department of Poultry Science, College of Agriculture, Auburn University), and Michael W. Pariza, Ph.D. (Professor and Director Emeritus, Food Research Institute, University of Wisconsin Madison; Michael W. Pariza, Consulting, LLC). The GRAS Panel critically evaluated Exponent's safety documentation (the dossier) as well as any other available data and information that the members of the GRAS Panel believed to be pertinent to the safety of the proposed use of berberine.

On November 18, 2020, the GRAS Panel convened via teleconference and independently, jointly, and unanimously concluded that berberine, produced consistent with current good manufacturing practice (cGMP) for animal feeds and meeting the stated specifications, is safe as a preservative in feed for broiler chickens. The GRAS Panel further concluded unanimously that the intended use of berberine as a preservative in feed for broiler chickens is GRAS based on scientific procedures. It is also the unanimous consensus opinion of the GRAS Panel members that other qualified experts would concur with their conclusions.

Summarized below are the data, information, and interpretive analysis supporting the GRAS Panel's conclusions.

Description

The subject of this review is berberine, with a purity of NLT 97.0% berberine (anhydrous basis). The chemical name is 5,6-Dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-α] quinolizinium chloride hydrate. It is extracted from dry bark of *Phellodendron chinense* with a hot solvent mixture of (b) (4) and (b) (4) and is produced in accordance with cGMP for animal feed). Berberine meets appropriate animal food-grade specifications. Analytical data from non-consecutive batches demonstrate the product specifications are consistently met.

Intended Use and Estimated Daily Intake

Berberine is intended for use as a preservative in feed for broiler chickens. Berberine will be added to the feed at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold.

Feed intake by poultry varies due to many factors such as size, age, gender, breed, environment, level of egg production, and quality of feed. Based on the proposed inclusion rate of berberine in feed of 0.03 g/kg (or 0.03 mg/g), the daily intake of berberine for male and female broilers are 6.12 mg/day and 4.95 mg/day, respectively.

Results from a poultry feeding study showed that at the "low" berberine diet concentration of 0.03 g/kg, the proposed maximum use level, berberine residues in chicken tissues were below the limit of detection (LOD) of 2 ng/g. Assuming the maximum residue level in broiler chicken tissues at the LOD (2 ng/g) and the intake of chicken food products (muscles, tissues and organs) based on US NHANES 2013-2016 food consumption data, the 90th percentile estimated daily intake (EDI) of berberine from the proposed use is 0.0000091 mg/kg bw/day and 0.0000039 mg/kg bw/day for children aged 2-5 y and the US population aged 2+y, respectively.

Assessment of Safety

Chicken Safety

Orally administered berberine was poorly absorbed in chickens, as indicated by the low absolute bioavailability of 2.03% and Cmax of 4.09 µg/l. Intravenously administered berberine resulted in rapid absorption, distribution, and elimination of berberine. Berberine was not detected (LOD of 2 ng/g) in tissues of chickens fed a low level of berberine in their diet (0.03g berberine/kg feed). Feeding studies showed no safety concern in chickens fed with berberine containing feed at concentration up to 1 g/kg. IRP Health's proposed maximum use level of berberine in chicken feed is 0.03g/kg and is, therefore, safe for broiler chickens.

Human Safety

Berberine has been assessed in acute, subchronic, chronic/carcinogenicity, genotoxicity, developmental and target animal safety studies as a component of goldenseal. These studies of the goldenseal product containing 3.45-3.89% berberine and the fibrous root of *Rhizoma Coptidis* containing 1.20% berberine provide indirect information regarding potential berberine toxicity. The studies can be used to assess berberine by assuming, as a worst case, that all of the toxicity in the studies is due to berberine. Other isoquinoline components of goldenseal, however, include hydrastine (2.0%) and canadine (0.5%) and these alkaloids are expected to contribute to goldenseal toxicity. Likewise, the fibrous root of *Rhizoma Coptidis* was determined to contain coptisine (1.28%), epiberberine (0.53%), and palmatine (0.08%) (Ning et al., 2015). Based on the highest 90th percentile intake among children 2-5 years (9.1 E-6 mg/kg bw/day) and comparing to the available subchronic no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) for berberine, the margin of exposures (MOE) are very large, > 100,000.

Comparison of the maternal NOAELs in developmental toxicity studies for berberine tested as a component of goldenseal and alone confirms the significant probability that other components of goldenseal contribute significantly to the toxicity of the mixture. Risk assessment based on the assumption that berberine content is responsible for the toxicological effects seen in studies of goldenseal greatly overestimates berberine risk.

An increased incidence of hepatocellular tumors was found in male and female rats receiving diet containing goldenseal with a 3.89% concentration of berberine. The International Agency for Research on Cancer (IARC) classified goldenseal root powder as "possibly carcinogenic to humans (Group 2B). The tumor response was found in a single site (liver) in a single species (rat). Although one carcinoma was seen in one treated male rat at the high dose level, only benign tumors were clearly treatment-related.

Berberine is not mutagenic but it inhibits topoisomerase, a category of DNA repair enzymes. Berberine also has antiproliferative properties *in vitro*. Dietary exposure to the target species and humans is low. The tumor dose-response of goldenseal suggests nonlinearity and a threshold of 15.75 mg/kg bw/day or greater. Based on the highest 90th percentile intake among children aged 2-5 years (9.1E-06 mg/kg bw/day), this threshold dose is 1,700,000-fold greater than the estimated dietary exposure from the consumption of chicken.

Although berberine is associated with an increase in benign liver tumors in the rat when tested as a component of the goldenseal root powder, these effects may be secondary to changes in liver function or due to other components of the goldenseal mixture. The absence of berberine induced genotoxicity or developmental toxicity suggests that berberine is not a direct acting carcinogen[1].

Reports of gastrointestinal-related adverse events have been summarized in published systematic reviews and meta-analyses. In a meta-analysis of 27 clinical trials on the effect and safety of berberine in the treatment of type 2 diabetes, hyperlipidemia and hypertension, 12 trials reported a number of adverse reactions and eight others stated slight adverse reactions without clear data. The statistical analysis showed that the incidence of toxic side effects is related to doses of berberine. At 600 mg berberine, the incidence rate of toxic side effects is 5%; when berberine dose is in the range of 900 to 1500 mg, the incidence rate is greater than 10% (Lan et al., 2015). The highest 90th percentile EDI for berberine from chicken consumption among children 2-5 years (0.00015 mg/day) is orders of magnitude below these observed adverse effect levels, and thus, not a safety concern.

Antimicrobial Resistance Risk

A hazard characterization following the FDA's Guidance for Industry (GFI) #152 was carried out to consider the theoretical concern that antimicrobial resistance could develop among zoonotic, bacterial pathogens of human health concern associated with the proposed use of berberine as a preservative in poultry feed. Berberine is not a typical antimicrobial and does not belong in any standard antimicrobial groupings. Berberine has shown a range of antimicrobial mechanisms. It causes cell membrane damage, cell membrane permeability changes and efflux pump inhibition. Interference with efflux pumping was observed to increase the lethality of other antibacterial agents. Evidence was also presented that berberine affects DNA/RNA and protein synthesis. Literature reports activity against both Gram positive (MRSA) and Gram

[1] The etiologies of developmental toxicity have many common pathways with carcinogenicity. For example, epigenetic changes induced *in utero* may increase susceptibility to cancer in aging animals (Ho et al., 2017; Cheong et al. 2018). Cytotoxicity and alterations in rates of cell division may be early events in both carcinogenicity and developmental toxicity.

negative bacteria, and the ability of berberine to inhibit growth was determined against many bacteria. Berberine is not on FDA's antimicrobial drug list. The primary human pathogens associated with poultry are *Salmonella* and *Campylobacter*, and berberine is not a treatment drug for humans for these pathogens. No studies were found showing the development of resistance to berberine by pathogenic bacteria. Furthermore, berberine appears to have a non-specific interaction with various cellular moieties in the membrane, nucleic acids and proteins and is, thus, not expected to lead to development of specific antimicrobial resistance mechanisms.

Summary and Conclusion

Berberine is intended for use as a preservative in feed for broiler chickens. It is proposed to be added to feed at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold. Berberine is produced in accordance with cGMP for animal feed and meets appropriate animal food-grade specifications.

Therefore, the intended use of berberine as a preservative in feed for broiler chickens at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold meets the FDA safety standard of a reasonable certainty of no harm under the conditions of intended use, and is therefore safe. The pivotal data and information upon which this conclusion is based are publicly available, and generally known and generally accepted by the broader scientific community of experts qualified by experience and training to evaluate the safety of food ingredients for use in human food and animal feed. Thus, the intended use of berberine as a preservative in feed for broiler chicken as described is also GRAS, based on scientific procedures.

GRAS Panel Conclusion

We, the members of the GRAS Panel, have individually and collectively critically evaluated the published and unpublished data and information summarized above, and conclude that the intended use of berberine, produced consistent with cGMP for animal feed and meeting animal food-grade specifications, as a preservative in feed for broiler chickens, at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold, is safe and suitable.

We, the members of the GRAS Panel, further conclude that the intended use of berberine, produced consistent with cGMP and meeting animal food-grade specifications, as a preservative in feed for broiler chickens, at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold, as presented in the supporting dossier is GRAS based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

(b)(6)

Dec 30, 2020

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Date

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Date

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Professor and Director Emeritus, Food Research Institute
University of Wisconsin Madison
Member, Michael W. Pariza, Consulting, LLC
Madison, Wisconsin

Date

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It is our opinion that other qualified experts would concur with these conclusions.

_____ _____

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Date

(b)(6) _____ 1-10-2021

William Dozier, Ph.D.
Professor, Department of Poultry Science
College of Agriculture
Auburn University

Date

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Michael W. Pariza, Ph.D.
Professor and Director Emeritus, Food Research Institute
University of Wisconsin Madison
Member, Michael W. Pariza, Consulting, LLC
Madison, Wisconsin

Date

GRAS Panel Conclusion

We, the members of the GRAS Panel, have individually and collectively critically evaluated the published and unpublished data and information summarized above, and conclude that the intended use of berberine, produced consistent with cGMP for animal feed and meeting animal food-grade specifications, as a preservative in feed for broiler chickens, at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold, is safe and suitable.

We, the members of the GRAS Panel, further conclude that the intended use of berberine, produced consistent with cGMP and meeting animal food-grade specifications, as a preservative in feed for broiler chickens, at a maximum concentration of 0.03 g/kg to preserve the feed from yeast and mold, as presented in the supporting dossier is GRAS based on scientific procedures.

It is our opinion that other qualified experts would concur with these conclusions.

William Allaben, Ph.D., ATS
Adjunct Professor, College of Medicine
Adjunct Professor, College of Public Health
University of Arkansas for Medical Sciences
Principal and Managing Toxicologist
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January 7, 2021

Date

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September 14, 2021

BY E-MAIL

Carissa Adams, MPH, Animal Scientist,
Division of Animal Feeds (HFV-220)
Center for Veterinary Medicine
U.S. Food and Drug Administration
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Rockville, MD 20855

Subject: Request to cease evaluation of GRAS Notice AGRN 47

Dear Ms. Adams:

On behalf of IRP Health Pty Ltd., I request that CVM cease evaluation of GRAS Notice AGRN 47.

Please let me know if you have any questions or need further information.

Sincerely

(b)(6)

Riëtte van Laack

RVL/eam