Available online at www.sciencedirect.com
ScienceDirect
Food and Chemical Toxicology 45 (2007) 1719–1730
www.elsevier.com/locate/foodchemtox

Toxicological assessment of a particulate yeast (1,3/1,6)-β-D-glucan in rats

K. Babíček [a], I. Čechová [a], R.R. Simon [b], M. Harwood [b,*], D.J. Cox [c]

[a] *BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, 254 49 Jilove, Czech Republic*
[b] *Cantox Health Sciences International, 2233 Argentia Road, Suite 308, Mississauga, ON, Canada L5N 2X7*
[c] *Biothera, Inc., 3388 Mike Collins Drive, Eagan, MN 55121, USA*

Received 16 November 2006; accepted 9 March 2007

Abstract

This study investigates the toxicity of WGP® 3–6, a yeast-derived β-glucan ingredient, during single-dose acute and sub-chronic toxicity studies in rats. For the acute study, Fisher-344 rats were administered WGP® 3–6 via gavage at a dose of 2000 mg/kg body weight, and any evidence of toxicity was monitored over a 14-day period. WGP® 3–6 was well tolerated, indicating that the LD_{50} value is greater than 2000 mg/kg body weight. For the sub-chronic study, Fisher-344 rats (10/sex/group) were randomly allocated to receive daily gavage treatment with WGP® 3–6 at doses of 0, 2, 33.3, or 100 mg/kg body weight. Control and high-dose satellite recovery groups of each sex also were included. Full toxicological monitoring and endpoint investigations were performed throughout and upon completion of the study. No negative effects on animal weights or food consumption attributable to WGP® 3–6 were evident at any dose. In addition, no mortality, clinical pathology, functional/behavioral, microscopic, or gross observations indicating toxicity were observed. Sporadic changes in some biochemical and hematological parameters were observed; however, since the effects were within the physiological ranges in historical controls, were not dose–responsive, or were not observed in both sexes, they were determined to be of no toxicological significance. In conclusion, no adverse or toxic effects were observed after subchronic oral administration of 2, 33.3, or 100 mg/kg body weight/day of WGP® 3–6 in Fisher-344 rats, and therefore, a no observed adverse effect level (NOAEL) of 100 mg/kg body weight/day, the highest dose tested, was determined.

Keywords: (1,3)-β-D-Glucan; Rat; Toxicity; Safety; Yeast; NOAEL

1. Introduction

1,3-β-D-Glucans are a group of highly conserved carbohydrate biopolymers that form a fibrous structural extracellular matrix within the cell walls of yeast and bacteria. In addition to yeast and bacteria, large quantities of 1,3-β-D-glucans also occur naturally in a number of edible mushrooms, specifically the Shitake (*Lentinus edodes*), Maitake (*Grifola frondosa*), Wood cauliflower (*Sparassis crispa* Fr.), and snow mushroom (*Tremella fuciformis*) varieties (Ko and Lin, 2004). Of non-fungus-derived food sources, it has been reported that celery, chi-chian leaves, carrot, and radish contain nearly 20% 1,3-β-D-glucan in their total carbohydrate fraction, and up to 0.8% of

Abbreviations: ALP, alkaline phosphatase; ALT, 2-oxoglutarate aminotransferase; ANOVA, analysis of variance; aPTT, activated partial thromboplastin times; AST, L-aspartate, 2-oxoglutarate aminotransferase; CR3, component 3 receptor; EDTA, ethylenediaminetetraacetic acid; USFDA, US Food and Drug Administration; GGT γ-glutamyl transferase; GLP, good laboratory practice; Hb, hemaglobin; Hct, hematocrit; K, potassium; LDL, low density lipoprotein; MCV, mean corpuscular volume; Na, sodium; NOAEL, no observed adverse effect level; NOEL, no observed effect level; OECD, Organization for Economic Co-operation and Development; PAMP, pathogen-associated molecular patterns; PRR, pattern recognition receptors; PT, prothrombin times; PTT, partial thromboplastin times; RBC, red blood cells; SPF, specific pathogen free; TLR, toll-like receptors; WBC, white blood cells; WHO, World Health Organization.

* Corresponding author.
E-mail address: jvowles@cantox.com (M. Harwood).

0278-6915/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2007.03.013

soybean dry weight contains 1,3-β-D-glucan (Ko and Lin, 2004).

Due to their highly conserved nature and extracellular location in yeast and bacteria, mammalian organisms have developed immune recognition systems for a number of extracellular β-glucan components. These microbial-derived glucan polymers, termed pathogen-associated molecular patterns (PAMP), are recognized by pattern recognition receptors (PRR) on immune cells. The specific PRR that bind 1,3-β-D-glucans are the toll-like receptors (TLR) and the non-opsonic receptor, Dectin-1 (Brown et al., 2003; Gantner et al., 2003; Herre et al., 2004; Dillon et al., 2006). In addition, 1,3-β-D-glucans also have been shown to bind the complement component 3 receptor (CR3) in neutrophils (Xia et al., 1999; Cramer et al., 2006).

The capacity of 1,3-β-D-glucans to interact with and modulate the immune system has stimulated widespread research into a variety of potential pharmaceutical and nutraceutical uses for these compounds. For example, 1,3-β-D-glucans isolated from *Saccharromyces cerevisiae* have been shown to inhibit cancer growth and enhance the anti-tumor effects of monoclonal antibodies in mice (Cheung et al., 2002; Vetvicka et al., 2002; Hong et al., 2004), to enhance neutrophil recovery following sub-lethal irradiation (Gu et al., 2005), to protect against anthrax infection (Vetvicka et al., 2002), and to attenuate microbial infections in mice, rats, and guinea pigs (Onderdonk et al., 1992; Cisneros et al., 1996; Kernodle et al., 1998; Hong et al., 2004; Breivik et al., 2005). Improved humoral and cellular immunity also have been demonstrated in weanling piglets consuming *S. cerevisiae*-derived β-glucan (Li et al., 2005). In humans, yeast-derived 1,3-β-D-glucans have been shown to protect against post-operative infection in high-risk surgical patients (Babineau et al., 1994a,b; Dellinger et al., 1999), to improve macrophage function in trauma patients (Browder et al., 1990), and to decrease plasma lipid levels following oral supplementation (Nicolosi et al., 1999).

Although the molecular structures of the various β-glucan chains (1,3, 1,6) are highly conserved, the three-dimensional structure of the molecule can vary dramatically among organisms and depends on the chain length, branch type, and branch frequency of the particular β-glucan. In addition, variations in β-glucan structure and composition are highly dependent on the particular method by which a β-glucan preparation is processed or manufactured, and these variations can have a significant impact on their respective immune stimulatory activity (Seljelid et al., 1981; Saitô et al., 1991; Bohn and BeMiller, 1995; Mueller et al., 2000; Willment et al., 2001), an effect that also could lead to significant toxicity when crude preparations are used therapeutically (Riggi and Di Luzio, 1961; Di Luzio et al., 1979; Bowers et al., 1986; Miura et al., 2003).

Using a proprietary technology, Biothera, Inc. (Eagan, MN) has isolated a highly pure, insoluble particulate β-glucan fraction, WGP® 3–6, from the cell wall of *S. cerevisiae,* which is more commonly known as bakers yeast. Some ingredients derived from bakers yeast (i.e., bakers yeast protein, glycan, and extract) are approved by the US Food and Drug Administration (US FDA) for use in foods (US FDA, 2006), and *S. cerevisiae*-derived glucans also are currently marketed in the US in a number of dietary supplement products. WGP® 3–6 is produced using acid and alkaline extraction techniques that result in the stripping of the outer mannoprotein sheath and loss of the inner cellular lipids and proteins, leaving a relatively intact β-D-glucan shell (Fig. 1). While WGP® 3–6 has been consumed by humans as a dietary supplement without reports of adverse effects, and also has been used in clinical trials with no observed toxicity, the safety of long-term WGP® 3–6 exposure has not been evaluated. Although a few pre-clinical safety assessments have been performed on β-glucans

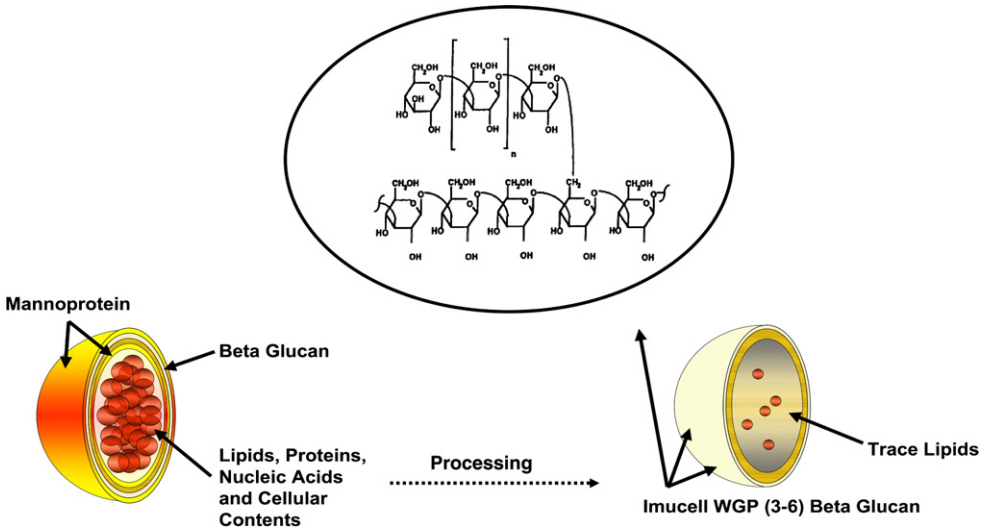

Fig. 1. Structure of WGP® 3–6 β-D-glucan shell. β-glucan is extracted from *Saccharromyces cerevisiae* using a proprietary technology, producing a whole glucan particle in which the outer surface of mannoprotein and inner cellular contents are removed to expose the β 1,3/1,6 glucan (WGP® 3–6). Also depicted is the molecular structure of a β-1,3 glucan chain linked through a 1,6 branch point.

derived from *S. cerevisiae* and *Candida albicans* (Williams et al., 1988; Feletti et al., 1992), as mentioned, β-glucan composition can vary significantly depending on the β-glucan preparation method. Therefore, as part of extensive safety investigations to support the use of WGP® 3–6 as a food ingredient, we are reporting the results of acute and sub-chronic toxicity studies in rats. The studies were performed under Good Laboratory Practice (GLP) conditions according to the Organization for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals (OECD, 2001) and the ingredient was tested using a range of oral doses for establishing a safe level for use in food.

2. Materials and methods

2.1. Test material

The test material used in the acute and subchronic toxicity studies was WGP® 3–6 [Lot #20030731 (acute study) and Lot #1033-003 (subchronic study); Biopolymer Engineering, Eagan MN]. WGP® 3–6 powder is a highly purified extract of *S. cerevisiae*, manufactured to the specifications shown in Table 1.

2.2. Acute toxicity study

2.2.1. Study design

A single-dose oral study in rats was conducted at the Japanese Food Research Laboratory (TAMA Laboratory, Nagayama, Japan) to evaluate the potential toxicity of high-dose WGP® 3–6. Ten (10) Brl-Han:WIST@Jcl rats were obtained from Japan CLEA Inc. at 5 weeks of age. Animals were acclimatized for 1 week to ensure that no abnormalities in animal condition existed. The rats were housed five animals per cage under standard laboratory conditions (temperature: 23 ± 2°C; relative humidity: 30–70% (target of 50–60%) light-dark cycle: 12/12 h) and received a standard diet (Nihon Nosankogyo K.K) and tap water ad libitum. Five male and five female rats were allocated to control and treatment groups, and base-line weights were recorded following a 17-h fasting period. WGP® 3–6 (Lot # 20030731) was formulated as a 100 mg/mL suspension and administered to each animal at a dose of 2000 mg/kg body weight (20 mL/kg body weight) by oral gavage. Control animals received equal volumes of water via the same administration route, and both groups were then observed for a period of 14 days. Clinical observations were made frequently on the day of administration and once a day during the 14-day observation period. The rats were weighed on days 7 and 14 following oral gavage, and the mean body weight values of the experimental and control groups were statistically analyzed using the *t*-test ($p = 0.05$). Experimental design was performed in accordance with OECD Guideline No. 420 (OECD, 2001).

Table 1
Product specification for WGP® 3–6

Attribute	Specification
Origin	*Saccharomyces cerevisiae*
β-Glucan 1,3/1,6	>75%
Carbohydrates	>80%
Protein	<3.5%
Fat	<12%
Ash	<3%
Moisture	<8%
Lead	<5 ppm
Aerobic Plate Count	<20,000 CFU/g
Coliform	<10 CFU/g
Salmonella sp.	Negative
Escherichia coli	Negative
Yeast and mold	⩽ 25 CFU /g combined

CFU = colony forming units; ppm = parts per million.

2.3. Sub-chronic toxicity study

2.3.1. Study design

Groups of 10 male and 10 female specific pathogen free (SPF) Fisher-344 rats were randomized to 1 of 4 groups receiving 0 (control), 2, 33.3, or 100 mg/kg body weight/day of WGP® 3–6 powder for 91 consecutive days by oral gavage. Separate control and 100 mg/kg body weight/day recovery groups for each sex also were included, and were maintained for an additional 14 days without treatment following the 91-day dosing period. Clinical observations, body weights, and food consumption were monitored throughout the treatment period, and at the selected termination dates (92 or 106 days) the animals were euthanized and subjected to a full post-mortem examination. The study was conducted at the Research Institute of Biopharmacy and Veterinary Drugs (Pohoří-Chotouň, Czech Republic) in compliance with the OECD Principles of GLP and in accordance with the OECD Guideline No. 408 for the Testing of Chemicals, "Repeated Dose 90-day Oral Toxicity Study in Rodents" (OECD, 2001).

2.3.2. Experimental animals and housing

One-hundred and sixty (160; 80 males and 80 females) SPF Fisher CDF (F-344)/CrlBR rats were obtained from Charles River Deutschland (via AnLab Ltd, Prague, Czech Republic). The animals were acclimatized for 13–16 days, and were 5–6 weeks of age and weighed between 80 and 100 g at the initiation of treatment. Sixty (60) males and 60 females were randomly selected according to weight criteria and allocated to the control and treatment groups. Rats were housed as groups of five animals per cage in standard plastic cages. The temperature inside the experimental room was kept at 22°C (±2°C) and the relative humidity was maintained at 30–70% (with an aim of 50–60%). Additional environmental controls involved the use of artificial lighting (12 h light/12 h dark), controlled air changes (>15 per hour), and microbiological environmental monitoring. Sterilized litter was inspected daily and changed 3 times a week. Animals received sterilized standard diets [ST-1; Borland, Kocanda, Czech Republic] and sterilized drinking water ad libitum.

2.3.3. Test item preparation

WGP® 3–6 was freshly suspended in sterile injection water everyday, and a constant volume of 0.5 mL per 100 g body weight of the test item suspension was orally administered by gavage on a daily basis. The concentration of the test item in the administration form was regularly analyzed for quality control purposes, the doses were set on the basis of the most recently recorded body weight of each individual animal, and the total amount of the test item administered was individually recorded.

2.3.4. Clinical examinations

Animals were observed twice daily for mortality and a general clinical examination was performed once a day. Detailed clinical observations were made in all animals prior to the first exposure to the test item and once a week thereafter. All signs of toxicity or morbidity were recorded.

Ophthalmic examinations were made on all animals prior to the first administration of control or WGP® 3–6 test article, at the end of the administration period, and at the end of the recovery period. External inspection of the eyes and peribulbar structures was performed macroscopically. Direct and indirect ophthalmic examinations were made using an ophthalmoscope (Maifield, Gowllands, UK), and animals were sedated using light halothane anesthesia. Short-acting mydriatic tropicamide (0.5%) in Mydriacyl ophthalmic drops (Alcon-Couvreur) was topically applied before performance of fundoscopy.

2.3.5. Functional and behavioral investigations

Towards the end of the administration period, a number of behavioral tests were conducted. For assessment of neuromuscular function, a beam

walking and bar grip test was performed; for determination of emotionality, ambulatory activity, and inquisitiveness, the open field test was used; and for assessment of visual function, the placing response test was performed (OECD, 2001).

2.3.6. Clinical pathology

2.3.6.1. Hematological examinations. Standard hematological tests for erythrocyte count (RBC) (10^{12}/L), hemoglobin (Hb) concentration (g/dL), hematocrit (Hct) index (%), mean corpuscular volume (MCV) (fL), and leukocyte count (WBC) (10^9/L) were conducted using an AL veterinary analyzer (Cell Counter, Model 2000; AL systeme, Germany). Differential leukocyte count (%) was conducted using panoptical staining of blood smears and counting via a standard microscope (WL-89; Opton). Platelet count (10^9/L) and blood clotting time also were measured.

2.3.6.2. Biochemical examination of blood. Clinical biochemical analysis of blood was conducted before dosing commencement, at the end of the administration period, and at the end of the recovery period in all treated animals. The plasma was separated via centrifugation and then frozen and transferred to the Imumed laboratory for analysis. The following parameters were measured using automatic biochemical analyzers (OPTIMA and Konelabe 60i, KONE, FINLAND): sodium (Na), potassium (K), albumin, cholesterol, creatinine, gamma-glutamyl transferase (GGT), urea, L-alanine, 2-oxoglutarate aminotrasferase (ALT), L-aspartate, 2-oxoglutarate aminotransferase (AST), glucose, total protein, alkaline phosphatase (ALP), bilirubin, triglyceride, and low density lipoprotein (LDL)-cholesterol. For LDL-cholesterol, initially LDL was selectively blocked, while lipoproteins other than LDL were oxidized via enzymatic route. The LDL was then released and selectively determined as a color product on numerous enzymatic reactions at 600 nm. HDL-cholesterol was not measured.

2.3.7. Pathology examinations

2.3.7.1. Gross pathology. Prior to post-mortem examination, all animals were weighed and thereafter sacrificed via CO_2 inhalation, with subsequent bleeding from the *vena cava caudalis*. All of the animals were submitted to full necropsy procedures, including the examination of the following organs and/or sites: external surface of body; all orifices; cranial cavity; cervical tissues and organs; thoracic cavity and its contents, organs, and tissues; and the abdominal cavity and its contents, organs, and tissues.

2.3.7.2. Organ weights. Immediately after dissection, the harvested organs were trimmed of any adherent tissues and weighed in closed Petri dishes in order to avoid drying effects. Wet weight was determined for the following organs: liver, brain, heart, kidneys, spleen, adrenals, testes, epididymides, uterus, ovaries, and thymus. Paired organs (kidneys, adrenal, testes, epididymides, ovaries) without visible pathological changes were weighed together. Organ weights were expressed as absolute values (g) and as relative (''organ to body weights'') values (g per 100 g of body weight).

2.3.7.3. Histopathology. Full histopathology was carried out on the organs and tissues of all animals in the control and the highest dosage (i.e., 100 mg WGP® 3–6/kg body weight/day) groups. Histological examinations were extended to animals of lower dosage groups if compound-related changes were observed in the high-dose group. Histopathology in recovery animals was performed on tissues and organs identified as showing effects in treated groups. With an aim of possible histological examination, samples of the following organs and tissues were collected from all experimental animals: brain, medulla and pons, cerebrum, cerebellum, spinal cord at three segments (cervical, mid-thoracic, and lumbar), pituitary gland, thyroid, parathyroid, thymus, trachea, lungs, heart, aorta, salivary gland, liver, spleen, kidneys, adrenals, pancreas, gonads, uterus, accessory genital organs, skin, tongue, esophagus, stomach, small intestine (duodenum, jejunum, and ileum, including Peyer's patches), large intestine (cecum, colon, and rectum), urinary bladder, mesenterial and popliteal lymph nodes, female mammary gland, prostate, musculature, peripheral nerve, sternum with bone marrow, femur and knee joint, eyes, and Harderian gland.

2.3.7.4. Organ/tissue processing. All organs/tissues sampled were fixed in 10% neutral buffered formalin except the eyes and Harderian glands, which were fixed in Davidson's fluid, and the testes and epididymides, which were fixed in Bouin's solution. Standard dehydration procedures and embedding in paraplast were performed. Tissues were cut to a thickness of 4 μm. Slides were stained with hematoxylin and eosin. Bones were decalcified with ethylenediaminetetraacetic acid (EDTA).

2.3.8. Statistical procedures

Means and standard deviations of means for groups, mean values of body weights, food consumption, absolute organ weights, relative organ weights, and hematological and biochemical parameters were calculated. Standard analysis of variance (ANOVA) was used for statistical evaluation of the data. When a significant dose-effect was found, further statistical analysis using Tukey's method and Dunnett's multiple comparisons were used for a detailed assessment of data obtained and for an assessment of their mutual relationship.

3. Results

3.1. Acute toxicity study

No deaths or clinical abnormalities for either male or female animals were observed during the experimental period. As shown in Table 2, in comparison to the control group, no significant differences in body weight gain occurred as a result of WGP® 3–6 administration. In addition, the findings of gross necropsy performed at study termination did not reveal any signs of toxicity. Under the conditions of this acute oral test, the LD_{50} value for WGP® 3–6 is greater than 2000 mg/kg body weight, the only dose tested.

3.2. Sub-chronic toxicity study

3.2.1. Body weights and food consumption

Mean body weights of males and females were measured weekly. As seen in Fig. 2, no significant differences in animal weights were observed between any of the treatment groups and the control groups. As shown in Fig. 3, no significant differences in mean weekly food consumption were observed between any of the groups, nor was any

Table 2
Average body weights of rats administered a single oral gavage dose of WGP® 3–6

Group	Body weight (g) (SD)		
	Pre-administration	7 days	14 days
Males			
Control (0 mg/kg bw)	145.5 (3.1)	192.0 (3.3)	235.7 (7.9)
2000 mg/kg bw	145.8 (2.1)	195.6 (4.3)	242.7 (4.2)
Females			
Control (0 mg/kg bw)	121.1 (4.5)	143.2 (7.5)	159.2 (12.5)
2000 mg/kg bw	121.3 (4.3)	144.4 (5.3)	163.9 (8.9)

bw = body weight; SD = standard deviation.

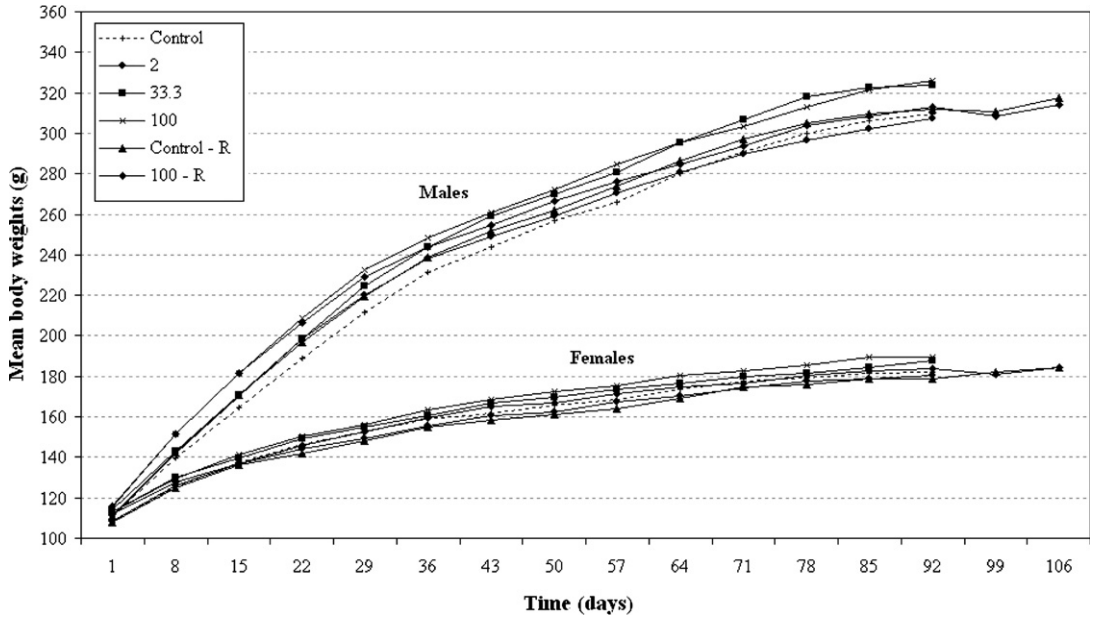

Fig. 2. Body weights of male and female rats given daily doses of WGP® 3–6 at the indicated doses by oral gavage for 91 days ($n = 10$ per group), followed by a 14-day recovery period in separate control and high-dose groups ($n = 10$ per group). The data represent the mean of each group.

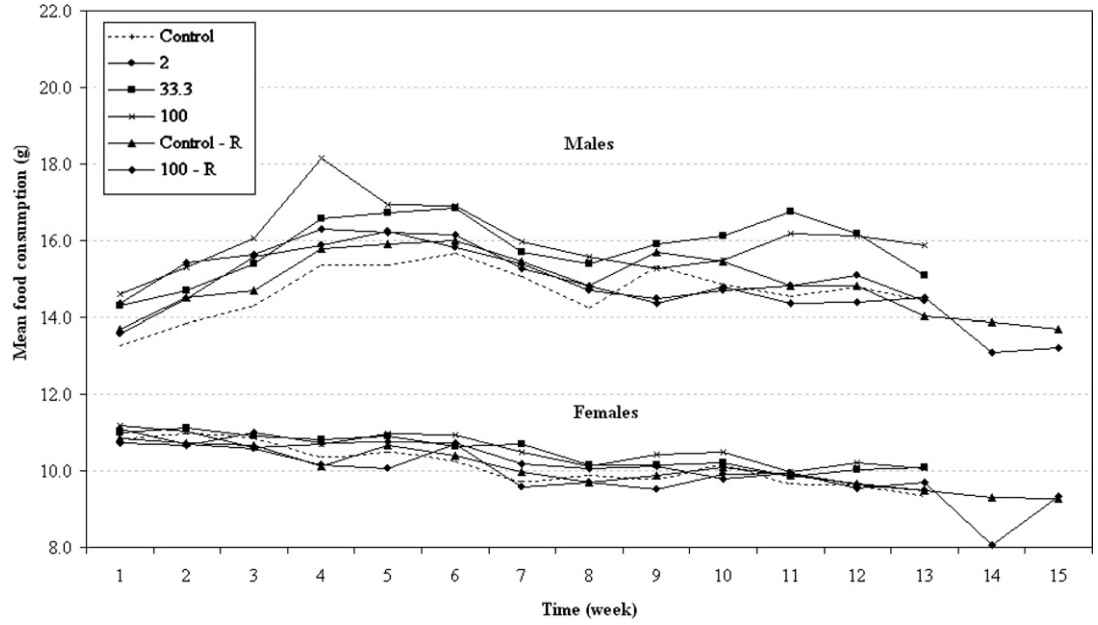

Fig. 3. Food consumption of male and female rats given daily doses of WGP® 3–6 at the indicated doses by oral gavage for 91 days ($n = 10$ per group), followed by a 14-day recovery period in separate high-dose and control groups ($n = 10$ per group). The data represent the mean of each group.

correlation observed between food consumption and the dose of WGP® 3–6.

3.2.2. In-life examination

The results of individual clinical examinations in male and female groups indicate that WGP® 3–6 was well tolerated throughout the treatment period, with no mortality or signs of morbidity observed. The only clinical change noted was moderate diarrhea and transient weight loss in one high-dose (100 mg/kg body weight/day) male during days 70–71. No evidence of diarrhea was observed in any other treated males, nor was diarrhea observed in any treated females; thus, the effect occurring in this single high-dose male was believed to be of no toxicological significance. There was no evidence of any compound-related effects on the results of the ophthalmic examinations.

A number of functional and behavioral tests were performed towards the end of the treatment period. Based

on the results of beam walking and bar grip strength experiments, no evidence of neuromuscular impairment was observed between control and WGP® 3–6-treated animals. In addition, no differences between treated rats and

Table 3
Summary of hematology values obtained in the subchronic oral gavage toxicity study of WGP® 3–6

Analysis at Day 92	Group	Dose group [dose (mg/kg body weight/day)]			
		Control [0]	Low-dose [2]	Mid-dose [33.3]	High-dose [100]
Males					
RBC ($\times 10^{12}$/L)	Mean	7.93	7.76	8.07	7.96[a]
	SD	0.19	0.24	0.15	0.22
MCV (fL)	Mean	44.4	44.8	45.5	46.2[a]
	SD	1.1	1.0	1.1	0.8
Hct (%)	Mean	35	35	37	37[a]
	SD	1	1	1	1
WBC ($\times 10^{9}$/L)	Mean	8.66	9.99	9.95	8.22
	SD	1.32	1.70	2.84	1.12
Hb (g/dL)	Mean	14.91	14.71	14.69	14.47
	SD	0.35	0.19	0.39	0.39
Segment neutrophils (%)	Mean	14.9	18.0	20.0	14.4
	SD	7.0	8.0	8.7	3.9
Band neutrophils (%)	Mean	0	0	0	0
	SD	0	0	0	0
Eo (%)	Mean	0.7	0.5	0.8	0.2
	SD	0.7	0.7	0.9	0.6
Ba (%)	Mean	0	0	0	0
	SD	0	0	0	0
Ly (%)	Mean	83.9	80.8	78.8	85.1
	SD	6.9	8.5	9.0	4.3
Mo (%)	Mean	0.5	0.7	0.4	0.3
	SD	0.5	0.8	0.7	0.5
PLT ($\times 10^{9}$/L)	Mean	532.1	606.9	538.2	533.0
	SD	23.5	93.1	85.9	31.3
Blood clotting (s)	Mean	141.1	100.4	93.9[a]	78.3[a]
	SD	68.5	24.7	25.2	20.9
Females					
RBC ($\times 10^{12}$/L)	Mean	7.52	7.67	7.45	7.47
	SD	0.28	0.21	0.17	0.18
MCV (fL)	Mean	44.6	48.7[a]	47.1[a]	47.6[a]
	SD	1.2	0.8	1.4	0.7
Hct (%)	Mean	34	37[a]	35	36
	SD	2	1	1	1
WBC ($\times 10^{9}$/L)	Mean	7.93	7.76	8.52	7.69
	SD	1.14	0.95	0.97	0.86
Hb (g/dL)	Mean	14.79	14.89	14.67	14.46
	SD	0.31	0.28	0.32	0.25
Segment neutrophils (%)	Mean	18.5	19.1	20.0	18.0
	SD	5.2	4.3	6.4	6.6
Band neutrophils (%)	Mean	0	0	0	0
	SD	0	0	0	0
Eo (%)	Mean	0.7	0.6	0.5	1.1
	SD	0.8	1.0	0.7	1.0
Ba (%)	Mean	0	0	0	0
	SD	0	0	0	0
Ly (%)	Mean	80.6	79.8	79.3	80.7
	SD	5.3	4.5	6.4	7.0
Mo (%)	Mean	0.2	0.5	0.2	0.2
	SD	0.4	0.5	0.4	0.4
PLT ($\times 10^{9}$/L)	Mean	561.5	633.6	581.9	627.8
	SD	44.9	64.5	52.5	56.4
Blood clotting (s)	Mean	89.0	98.1	74.9	90.9
	SD	36.9	29.3	25.9	43.3

Ba = basophil; Eo = eosinophil; fL = femtolitres; g/dL = grams/decilitre; Hb = hemoglobin; Hct = hematocrit; Ly = lymphocyte; MCV = mean corpuscular volume; Mo = monocyte; PLT = polymorphonuclear lymphocytes; RBC = erythrocyte count (red blood cells); SD = standard deviation; WBC = leukocyte count (white blood cells).

[a] Significantly different from the control group at 0.01 using Dunnett's test.

Table 4
Summary of serum chemistry values obtained in the subchronic oral gavage toxicity study of WGP® 3–6

Analysis day 92	Group	Dose group [dose (mg/kg body weight/day)]			
		Control [0]	Low-dose [2]	Mid-dose [33.3]	High-dose [100]
Males					
Creatinine (μmol/L)	Mean	34.90	32.50	27.60	32.80
	SD	3.11	3.34	2.91	3.29
Urea (mmol/L)	Mean	8.66	8.07	8.15	8.38
	SD	1.00	0.74	1.21	0.45
ALT (μkat/L)	Mean	1.576	1.552	1.504	1.623
	SD	0.566	0.482	0.247	0.311
AST (μkat/L)	Mean	1.483	1.416	1.706	1.556
	SD	0.259	0.271	0.485	0.285
Glucose (mmol/L)	Mean	8.05	7.50	8.36	7.59[a]
	SD	0.47	0.52	0.96	0.51
TP (g/L)	Mean	70.39	70.34	68.33	68.89
	SD	1.72	2.77	10.10	1.84
Bilirubin (μmol/L)	Mean	29.35	26.09	33.80	34.26
	SD	5.47	7.81	17.96	8.36
Na (mmol/L)	Mean	141.3	140.7[a]	140.2	140.7[a]
	SD	0.8	0.5	0.9	0.8
K (mmol/L)	Mean	3.90	3.95	3.92	3.88[a]
	SD	0.15	0.17	0.18	0.21
GGT (μkat/L)	Mean	0.035	0.042	0.040	0.022
	SD	0.035	0.031	0.024	0.026
Albumin (g/L)	Mean	39.50	37.52	36.07	37.68
	SD	0.65	4.11	5.41	1.66
Cholesterol (mmol/L)	Mean	1.59	1.75	1.92	1.50
	SD	0.13	0.36	1.28	0.15
LDL-Cholesterol (mmol/L)	Mean	0.298	0.340	0.421	0.318
	SD	0.034	0.084	0.206	0.042
TG (mmol/L)	Mean	1.960	2.070	3.020	2.100
	SD	0.313	0.658	2.923	0.356
ALP (μkat/L)	Mean	2.78	2.38	2.59	2.73
	SD	0.21	0.24	0.77	0.20
Females					
Creatinine (μmol/L)	Mean	35.30	32.90	31.60	36.40
	SD	3.77	2.88	5.27	3.34
Urea (mmol/L)	Mean	9.06	7.67[a]	9.46	9.11
	SD	1.05	1.35	1.07	0.73
ALT (μkat/L)	Mean	1.224	1.022	0.929	1.100
	SD	0.256	0.232	0.129	0.261
AST (μkat/L)	Mean	1.486	1.300	1.494	1.301
	SD	0.206	0.228	0.168	0.102
Glucose (mmol/L)	Mean	7.87	6.28[a]	7.82	7.71
	SD	0.62	1.44	0.66	0.42
TP (g/L)	Mean	66.53	62.13[a]	66.29	65.21
	SD	1.85	6.20	2.47	2.39
Bilirubin (μmol/L)	Mean	24.35	22.24	38.00[a]	22.12
	SD	4.45	17.95	10.35	9.40
Na (mmol/L)	Mean	145.7	143.0[a]	139.2[a]	139.7[a]
	SD	2.8	0.8	0.8	0.9
K (mmol/L)	Mean	3.77	3.70	3.79	3.62
	SD	0.22	0.22	0.14	0.18
GGT (μkat/L)	Mean	0.090	0.110	0.109	0.056
	SD	0.064	0.101	0.035	0.032
Albumin (g/L)	Mean	37.52	36.18	38.45	37.88
	SD	2.95	3.19	1.31	2.26
Cholesterol (mmol/L)	Mean	2.26	2.02[a]	2.39	2.25
	SD	0.15	0.22	0.21	0.17
LDL-Cholesterol (mmol/L)	Mean	0.531	0.465[a]	0.476	0.457
	SD	0.083	0.153	0.043	0.052
TG (mmol/L)	Mean	1.190	1.180	1.480	0.960
	SD	0.351	1.181	0.371	0.479
ALP (μkat/L)	Mean	4.06	1.91	2.25	2.34
	SD	6.13	0.42	0.26	0.35

ALP = alkaline phosphatase, ALT = L-alanine:2-oxoglutarate aminotransferase, AST = L-aspartate:2-oxoglutarate aminotransferase, GGT = γ-glutamyl transpeptidase; K = potassium, LDL = low-density lipoprotein, Na = sodium, TG = triglycerides, TP = total protein.

[a] Significantly different from the control group at 0.01 using Dunnett's test.

controls in emotionality, ambulatory activity, or inquisitiveness were observed in the placing response and open-field behavior tests.

3.2.3. Hematological and clinical chemistry examination

Data for the hematology and clinical chemistry are presented in Tables 3 and 4. Although sporadic, statistically significant, increases in RBC, MCV, and Hct occurred in males at the highest dose (100 mg/kg body weight/day), the differences were less than 10% and did not exceed the maximums of physiological and historical control data ranges. Also, samples from the corresponding recovery groups displayed a tendency for normalization of all of the above-mentioned parameters. Of particular interest, a dose-dependent and significant decrease in clotting times was observed in male animals, which decreased by 44% at the highest dose; however, since the decline was within the historical control range, was not present in both sexes, and returned to baseline levels following withdrawal of dosing, the effect was considered to be of no clinical significance. A statistically significant increase in MCV also was observed in females, although, the changes in RBC, Hct, and blood clotting times seen in males were not observed in the female test groups.

Similar to the hematological evaluation, isolated statistically significant changes were observed for some clinical chemistry parameters (bilirubin, glucose, Na, K, cholesterol, LDL-cholesterol, urea, total protein); however, since the changes were within historical control values, were not dose–responsive, and did not occur in both sexes, the

Table 5
Summary of absolute and relative organ weight values (g/100 g) obtained in the subchronic oral gavage toxicity study of WGP® 3–6

Day 92	Group	Dose group [dose (mg/kg body weight/day)][a]			
		Control [0]	Low-dose [2]	Mid-dose [33.3]	High-dose [100]
Males					
Final body weight	Absolute	309.5 (12.3)	307.5 (21.0)	323.9 (15.2)	326.0 (12.3)
Liver	Absolute	10.179 (0.607)	9.611 (0.917)	11.304 (0.918)*	10.670 (0.685)
	Relative	3.288 (0.117)	3.123 (0.167)	3.492 (0.262)	3.274 (0.182)
Spleen	Absolute	0.580 (0.039)	0.631 (0.078)	0.612 (0.026)	0.612 (0.029)
	Relative	0.188 (0.012)	0.205 (0.023)*	0.189 (0.013)	0.188 (0.010)
Kidneys	Absolute	2.078 (0.169)	2.127 (0.147)	2.261 (0.158)*	2.230 (0.150)
	Relative	0.671 (0.036)	0.692 (0.018)	0.698 (0.023)	0.684 (0.029)
Adrenal Glands	Absolute	0.047 (0.006)	0.048 (0.005)	0.050 (0.007)	0.056 (0.006)*
	Relative	0.015 (0.002)	0.016 (0.002)	0.015 (0.003)	0.017 (0.002)
Heart	Absolute	0.898 (0.064)	0.915 (0.068)	0.970 (0.043)*	0.950 (0.046)
	Relative	0.290 (0.017)	0.298 (0.013)	0.300 (0.014)	0.291 (0.013)
Thymus	Absolute	0.251 (0.018)	0.253 (0.044)	0.276 (0.041)	0.264 (0.035)
	Relative	0.081 (0.005)	0.082 (0.013)	0.085 (0.012)	0.081 (0.011)
Brain	Absolute	1.804 (0.054)	1.819 (0.047)	1.811 (0.077)	1.814 (0.045)
	Relative	0.584 (0.030)	0.593 (0.033)	0.560 (0.023)	0.557 (0.015)
Testes	Absolute	2.854 (0.140)	2.996 (0.136)	2.984 (0.141)	2.988 (0.106)
	Relative	0.923 (0.045)	0.977 (0.062)*	0.922 (0.039)	0.917 (0.032)
Epididymides	Absolute	0.905 (0.065)	0.916 (0.060)	0.926 (0.044)	0.900 (0.049)
	Relative	0.292 (0.017)	0.298 (0.017)	0.286 (0.013)	0.276 (0.017)
Females					
Final body weight	Absolute	182.2 (9.4)	180.6 (7.2)	187.8 (7.5)	189.4 (9.2)
Liver	Absolute	5.579 (0.419)	5.555 (0.521)	6.012 (0.341)	5.947 (0.736)
	Relative	3.064 (0.216)	3.079 (0.315)	3.201 (0.124)	3.142 (0.383)
Spleen	Absolute	0.412 (0.033)	0.409 (0.027)	0.425 (0.029)	0.436 (0.047)
	Relative	0.226 (0.016)	0.226 (0.014)	0.226 (0.015)	0.231 (0.026)
Kidneys	Absolute	1.340 (0.048)	1.358 (0.047)	1.368 (0.074)	1.436 (0.056)*
	Relative	0.736 (0.029)	0.753 (0.028)	0.728 (0.020)	0.759 (0.036)
Adrenal glands	Absolute	0.051 (0.005)	0.053 (0.006)	0.051 (0.004)	0.054 (0.005)
	Relative	0.028 (0.002)	0.029 (0.004)	0.027 (0.002)	0.028 (0.003)
Heart	Absolute	0.603 (0.036)	0.604 (0.023)	0.626 (0.032)	0.622 (0.032)
	Relative	0.331 (0.016)	0.334 (0.014)	0.333 (0.015)	0.329 (0.016)
Thymus	Absolute	0.241 (0.026)	0.199 (0.025)*	0.209 (0.028)*	0.221 (0.019)
	Relative	0.132 (0.014)	0.110 (0.011)*	0.111 (0.013)*	0.117 (0.007)*
Brain	Absolute	1.683 (0.023)	1.680 (0.046)	1.687 (0.041)	1.694 (0.035)
	Relative	0.926 (0.044)	0.931 (0.042)	0.899 (0.029)	0.896 (0.041)
Uterus	Absolute	0.454 (0.087)	0.426 (0.071)	0.512 (0.112)	0.477 (0.096)
	Relative	0.248 (0.039)	0.236 (0.039)	0.272 (0.056)	0.252 (0.052)
Ovaries	Absolute	0.104 (0.014)	0.097 (0.017)	0.106 (0.015)	0.106 (0.012)
	Relative	0.057 (0.008)	0.054 (0.009)	0.056 (0.007)	0.056 (0.007)

* Significantly different from the control group at 0.01 using Dunnett's test.
[a] Where the presented values represent the mean and the values within parentheses represent the standard deviation.

effects were considered to be of no toxicological significance.

3.2.4. Gross necropsy

No pathological findings were present in any animals subject to necropsy after termination of the recovery period. Infrequent findings of very fine nodular adhesions (approximately 1 mm in diameter) were detected on the spleen capsule of four treated animals: one male in the low-dose (2 mg/kg body weight/day) group; one male and one female in the mid-dose (33.3 mg/kg body weight/day) group, and one male in the-high dose group. Overall, gross necropsy examination of animal organs and tissues revealed no pathological alterations attributable to WGP® 3–6.

3.2.5. Organ weights

Absolute and relative organ weights are shown in Table 5. There were some slight (⩽10%) but statistically significant changes in the absolute and relative organ weights in treatment versus control groups. In males, a significant increase in the absolute weights of the liver, kidneys, and heart was recorded in the 33.3 mg/kg body weight/day group, and in the adrenal glands of the high-dose group. In females, an elevation of absolute weights of the kidneys in high-dose animals and a non-dose-dependent decrease of the absolute weight of the thymus in the low- and mid-dose groups was observed. These sporadic differences in organ weights were not connected with any pathologic processes, and no connection with potential effects of WGP® 3–6 was observed. All mean values of absolute weights were within the limits of historical variability.

A significant increase in relative weights of the spleen and testicles in comparison to the control group was observed in the group of males treated with WGP® 3–6 at a dose of 2 mg/kg body weight/day. A significant, non-dose-dependent decrease in the relative weights of the thymus was observed in all treated females in comparison to the control group, although this effect was not observed in males. No alterations in any of the organs indicating a relationship with effects of WGP® 3–6 were revealed by gross necropsy.

3.2.6. Histopathology

No increases in the incidence of histopathological findings were observed in the group treated with the highest dose of WGP® 3–6 compared to the controls. Marked nodular histopathological alterations on the spleen were identified in one control animal and were attributed to a sporadic incidence of malignant lymphoma in this animal. Histopathological lesions present in organs and tissues of other animals were prevailingly of a sporadic character, and no relationship between their incidence and effects of the test item or the level of the dose were observed. The findings occurring more frequently (calcifications in the female renal tubules, small hyaline casts in the male renal tubules, small foci of fibrous adhesions on the spleen capsule) were present randomly at similar frequencies in animals of both the control and treated groups.

4. Discussion

Overall, oral administration of WGP® 3–6 was well tolerated. An acute study in BrlHan:WIST@Jcl rats showed no evidence of toxicity over a 14-day observation period following single oral gavage administration of WGP® 3–6 at a dose of 2000 mg/kg body weight. Repeated oral dosing of WGP® 3–6 at doses of 2, 33.3, or 100 mg/kg body weight/day in Fisher CDF (F-344)/CrlBR rats for a period of 91 days resulted in no increase in mortality, morbidity, alterations of health status, or reductions in diet consumption, body weights, or weight gain. No pathological changes were detected by ophthalmic examination, nor were any toxicologically significant changes observed in biochemical or hematological parameters. Post-mortem examination did not reveal pathologic changes in organs and tissues that were related to administration of the test compound, and no correlation between the dose level of WGP® 3–6 and alterations in absolute and relative organ weights were observed.

The only clinical observation worth noting was the dose-dependent and significant decrease in blood clotting times noted in male animals (up to 44% at the highest dose). The statistical significance of this finding was likely a result of the fact that the clotting times were unexpectedly elevated in the control group. Additionally, all group means were within historical control values and there were no significant differences observed for this parameter at the end of the 14-day recovery period. Furthermore, similar changes in clotting times were not reported in females, and no significant differences or trends in platelet count were observed for any treatment group. In vitro observations of enhanced clot formation of human plasma were reported by Ohno et al. (1990) for yeast-derived β-(1,3)-glucans; however, the reference is an isolated report and no mechanistic evidence could be identified to explain the apparent pro-coagulant effect.

Moreover, the use of whole blood clotting times also is considered to be an insensitive measure of hemostasis (Brown, 1992), and in the absence of additional mechanistic measures of blood clotting such as prothrombin times (PT), partial thromboplastin times (PTT) or activated partial thromboplastin times (aPTT), a conclusion regarding the effects of WGP® 3–6 on hemostasis for male animals during this experiment is difficult to make. Decreased clotting times or other pro-coagulant effects have not been reported in other β-glucan toxicity studies (Williams et al., 1988; Feletti et al., 1992). In addition, in human clinical trials using both orally administered WGP® 3–6 and Biothera, Inc.'s soluble pharmaceutical grade glucan (PGG-glucan), decreased clotting times were not reported to occur (Babineau et al., 1994a,b; Dellinger et al., 1999; Nicolosi et al., 1999). Therefore, in the absence of decreased clotting times in female animals, and given no reports of yeast glucan effects

on clotting times in the literature and no indication of decreased clotting times in humans consuming large quantities of WGP® 3–6 in clinical trials (Nicolosi et al., 1999), the observation of decreased clotting times in male rats should be interpreted as a spurious finding of no toxicological significance.

Two other pre-clinical toxicity studies have been published regarding β-glucans extracted from yeast (Williams et al., 1988; Feletti et al., 1992). The study by Feletti et al. (1992) investigated the chronic toxicity of an insoluble β-glucan preparation derived from *C. albicans* in Sprague–Dawley male and female rats. The β-glucan composition was not well characterized in the published article; however, it was reported to be prepared using an acid–alkali–detergent treatment and contained less than 0.5% protein. The β-glucan preparation was prepared as a liquid suspension and administered by oral gavage at doses of 0 (control), 50, 100, or 200 mg/kg body weight/day for a period of 52 weeks. At the end of the study period, the authors reported that no test item-related morbidity or mortality was observed, and no differences between control or treatment groups were reported with regard to body weight gain, food consumption, water intake, opthalmic evaluations, hematology, blood biochemistry, urinalysis, or organ weight. The only significant effect observed was caecal enlargement with variable hyperplasia of the colon mucosa in high-dose (200 mg/kg body weight/day) animals, an effect that was reversible upon cessation of treatment. The authors reported a no observed effect level (NOEL) of 100 mg/kg body weight/day. As stated by Felletti et al. (1992), caecal enlargement is typical of exposure to sugar alcohols (sorbitol, mannitol, xylitol), lactose, caramel, and some chemically-modified food starches, such as synthetic polydextrose, and is a physiological change that is considered by the World Health Organization (WHO) to be of no toxicological concern with respect to extrapolation of animal data to humans (WHO, 1987). Therefore, it is reasonable to state that, in addition to the NOEL of 100 mg/kg body weight/day derived by the authors, a no observed adverse effect level (NOAEL) of 200 mg/kg body weight/day can be assumed.

In a pre-clinical safety evaluation by Williams et al. (1988), the authors investigated the effects of a soluble β-glucan preparation isolated from *S. cerevisiae*. Unfortunately, no information was available regarding the composition/purity of the β-glucan preparation other than that it was analyzed to ensure the absence of endotoxin. The authors reported that acute single administration of the soluble β-glucan preparation at intravenous doses of up to 1000 mg/kg body weight in male ICR/HSD mice had no effect on mortality, and no effect on Harlan Sprague–Dawley rats at a dose of 500 mg/kg body weight. Differential species-specific effects were observed during repeat intraperitoneal dosing, where soluble β-glucan administration (250 mg/kg body weight/day) over 7 days resulted in no effect on weight gain in male ICR/HSD mice, yet produced a significant 10% decrease in weight gain in Sprague–Dawley guinea pigs (Williams et al., 1988).

During chronic studies in male ICH/HSD mice, Williams et al. (1988) administered β-glucan intravenously at doses of 40, 200, or 1000 mg/kg body weight twice weekly in two separate experiments spanning 30 and 60-day periods. Significant toxicity leading to hepatosplenomegaly was observed in the 40 and 1000 mg/kg body weight dose groups during the 30-day study. A dose-dependent increase in the incidence of hepatosplenomegaly also was observed in the 60-day study, a finding attaining statistical significance at the 1000 mg/kg body weight dose. Reticuloendothelial toxicity and lung granuloma have been reported in other studies; however, these effects are generally confined to the use of insoluble β-glucans administered via intraperitoneal or intravenous routes (Riggi and Di Luzio, 1961; Di Luzio et al., 1979; Johnson et al., 1984; Bowers et al., 1986; Patchen and MacVittie, 1986). The reason why hepatosplenomegaly occurred in the study by Williams et al. (1988), with a soluble form of β-glucan, is unclear; however, production details of the β-glucan preparation were not reported. Given the highly insoluble nature of yeast β-glucan, it is possible that small amounts of β-glucan precipitates were present in the preparation. In addition, in the absence of proper quality control procedures, contamination with fatty acids, proteins, or nucleic acids also may have been a factor.

Based on observations of splenotoxicity in other studies, the observation of small focal nodular lesions on the spleen capsule randomly distributed among four animals in the treatment groups of some animals treated with WGP® 3–6, in some cases associated with the presence of macrophages and lymphocytes is of interest; however, the effect was not dose–responsive, and was not associated with dose–responsive increases in relative spleen weights or microscopic evidence of splenotoxicity. Furthermore, only one group of animals (males of the 33.0 mg/kg body weight/day group) had an increased relative spleen weight in comparison to controls, and the magnitude of the effect was modest at 8.0%, and the spleen weights of the individual animals with spleen adhesis also did not reveal any correlation to potential toxicologic effects. More importantly, histopathology revealed spleen adhesis in three males of the recovery control group and one male in the recovery treatment group, which strongly suggests that the presence of sporadic spleen adhesis is due to random biological variation and is of no toxicological significance. Moreover, these findings are consistent with the results of the study by Feletti et al. (1992), who did not observe any evidence of increases in spleen weights or histopathological effects at similar doses of yeast β-glucan administered by gavage over a period of 52 weeks.

An additional observation made during the current subchronic toxicology study was a significant non-dose-related decrease in the absolute thymus weights in females administered WGP® 3–6 at doses of 2 and 33.3 mg/kg body weight (~15%; $p < 0.01$), and in the relative thymus weights

in females of all groups (~15%; $p < 0.01$). For immune related endpoints, the absence of a dose–response relationship does not necessarily support the conclusion of a sporadic finding (Descotes, 2004); however, a number of factors suggest that the observed decreases in thymus weights are indeed spurious findings. Firstly, the overall magnitude of the effect was modest (~15%). There also was a lack of similar findings in male animals. No significant alterations in white blood cell or lymphocyte counts were observed, nor were any alterations in thymus histopathology for either sex identified. Moreover, no significant differences in thymus weights were observed in the female recovery groups. No effects on thymus weight were observed in the chronic study by Feletti et al. (1992), where yeast β-glucan was administered to rats via oral gavage over a period of 52 weeks. An abundance of animal and human data provide evidence that β-glucans possess potential immune-enhancing activity, and not immune-suppressing activity, which is typically observed with decreases in thymus weights. Therefore, the study results and the data of previous studies available in the published literature further support the conclusion that the observed decreases in thymus weights in the females of the treatment groups are of no toxicological concern.

The safe toxicologic profile of orally administered yeast β-glucans observed during the subchronic toxicity study is consistent with the fact that WGP® 3–6 is indigestible and not absorbed to a significant degree. Systemic exposure to WGP® 3–6 may occur through macrophage uptake in the Peyer's patches of the small intestine. β-Glucan particles are then degraded within the macrophage, and soluble fragments are released at various systemic sites (i.e., bone marrow, lymph nodes) where neutrophil priming can occur (Hong et al., 2004; Li et al., 2006). Since evidence of reticuloendothelial toxicity is primarily confined to animals receiving significant systemic exposure to insoluble β-glucans, or to high-dose intravenous infusion of soluble forms, overloading of the immune system would not be expected with oral consumption of insoluble WGP® 3–6, although it is possible that systemic exposure to WGP® 3–6 may have occurred in a few animals as a result of physical damage to the gastric mucosa from repetitive gavaging.

WGP® 3–6 is a highly pure, insoluble particulate β-glucan fraction from the cell wall of *S. cerevisiae*, otherwise known as bakers yeast. The consumption of *S. cerevisiae*-derived β-glucan has a long indirect history of safe use through the consumption of bakers yeast, and various purified forms of yeast-derived β-glucans are routinely consumed as dietary supplements with no evidence of adverse effects. This report is the first publication where a biologically active insoluble yeast-derived β-glucan has been extensively studied under GLP conditions and to OECD specifications. The safe use and absence of toxicity observed for oral WGP® 3–6 administration in this study is consistent with observations in previous acute pre-clinical efficacy studies using WGP® 3–6 in rodents (Vetvicka et al., 2002), and repeat administration of WGP® 3–6 in quantities as high 15 g per day have been used safely in humans (Nicolosi et al., 1999). In conclusion, based on clinical, pathological, and statistical evaluations, no toxicity was observed over the 13-week repeat administration period when WGP® 3–6 was administered at up to a maximum deliverable oral dose of 100 mg/kg body weight/day to rats. Therefore, the NOAEL for this study was determined to be 100 mg/kg body weight/day, the highest dose tested.

References

Babineau, T.J., Marcello, P., Swails, W., Kenler, A., Bistrian, B., Forse, R.A., 1994a. Randomized phase I/II trial of a macrophage-specific immunomodulator (PGG-glucan) in high-risk surgical patients. Ann. Surg. 220, 601–609.

Babineau, T.J., Hackford, A., Kenler, A., Bistrian, B., Forse, R.A., Fairchild, P.G., Heard, S., Keroack, M., Caushaj, P., Benotti, P., 1994b. A phase II multicenter, double-blind, randomized, placebo-controlled study of three dosages of an immunomodulator (PGG-glucan) in high risk surgical patients. Arch. Surg. 129, 1204–1210.

Bohn, J.A., BeMiller, J.N., 1995. (1→3)-β-D-Glucans as biological response modifiers: A review of structure–functional activity relationships. Carbohydr. Polym. 28, 3–14.

Bowers, G.J., Patchen, M.L., MacVittie, T.J., Hirsch, E.F., Fink, M.P., 1986. A comparative evaluation of particulate and soluble glucan in an endotoxin model. Int. J. Immunopharmacol. 8, 313–321.

Breivik, T., Opstad, P.K., Engstad, R., Gundersen, G., Gjermo, P., Preus, H., 2005. Soluble β-1,3/1,6-glucan from yeast inhibits experimental periodontal disease in Wistar rats. J. Clin. Periodontol. 32, 347–352.

Browder, W., Williams, D., Pretus, H., Olivero, G., Enrichens, F., Mao, P., Franchello, A., 1990. Beneficial effect of enhanced macrophage function in the trauma patient. Ann. Surg. 211, 605–612, & discussion 612-613.

Brown, G., 1992. Hematology tests in toxicology: time for a re-think? Comp. Hematol. Int. 2, 231–235.

Brown, G.D., Herre, J., Williams, D.L., Willment, J.A., Marshall, A.S., Gordon, S., 2003. Dectin-1 mediates the biological effects of β-glucans. J. Exp. Med. 197, 1119–1124.

Cheung, N.-K.V., Modak, S., Vickers, A., Knuckles, B., 2002. Orally administered β-glucans enhance anti-tumor effects of monoclonal antibodies. Cancer Immunol. Immunother. 51, 557–564.

Cisneros, R.L., Gibson (III)., F.C., Tzianabos, A.O., 1996. Passive transfer of poly-(1–6)-β-glucotriosyl-(1–3)-β-glucopyranose glucan protection against lethal infection in an animal model of intra-abdominal sepsis. Infect. Immun. 64, 2201–2205.

Cramer, D.E., Allendorf, D.J., Baran, J.T., Hansen, R., Marroquin, J., Li, B., Ratajczak, J., Ratajczak, M.Z., Yan, J., 2006. β-glucan enhances complement-mediated hematopoietic recovery after bone marrow injury. Blood 107, 835–840.

Dellinger, E.P., Babineau, T.J., Bleicher, P., Kaiser, A.B., Seibert, G.B., Postier, R.G., Vogel, S.B., Norman, J., Kaufman, D., Galandiuk, S., Condon, R.E., 1999. Effect of PGG-glucan on the rate of serious postoperative infection or death observed after high-risk gastrointestinal operations (for the Betafectin Gastrointestinal Study Group). Arch. Surg. 134, 977–983.

Descotes, J., 2004. Importance of immunotoxicity in safety assessment: a medical toxicologist's perspective. Toxicol. Lett. 149, 103–108.

Di Luzio, N.R., Williams, D.L., McNamee, R.B., Edwards, B.F., Kitahama, A., 1979. Comparative tumor inhibitory and antibacterial activity of soluble and particulate glucan. Int. J. Cancer 24, 773–779.

Dillon, S., Agrawal, S., Banerjee, K., Letterio, J., Denning, T.L., Oswald-Richter, K., Kasprowicz, D.J., Kellar, K., Pare, J., van Dyke, T., Ziegler, S., Unutmaz, D., Pulendran, B., 2006. Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. J. Clin. Invest. 116, 916–928.

Feletti, F., De Bernardi di Valserra, M., Contos, S., Mattaboni, P., Germogli, R., 1992. Chronic toxicity study on a new glucan extracted from *Candida albicans* in rats. Arzneimittelforschung 42, 1363–1367.

Gantner, B.N., Simmons, R.M., Canavera, S.J., Akira, S., Underhill, D.M., 2003. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J. Exp. Med. 197, 1107–1117.

Gu, Y.-H., Takagi, Y., Nakamura, T., Hasegawa, T., Suzuki, I., Oshima, M., Tawaraya, H., Niwano, Y., 2005. Enhancement of radioprotection and anti-tumor immunity by yeast-derived β-glucan in mice. J. Med. Food 8, 154–158.

Herre, J., Gordon, S., Brown, G.D., 2004. Dectin-1 and its role in the recognition of β-glucans by macrophages. Mol. Immunol. 40, 869–876.

Hong, F., Yan, J., Baran, J.T., Allendorf, D.J., Hansen, R.D., Ostroff, G.R., Xing, P.X., Cheung, N.-K.V., Ross, G.D., 2004. Mechanism by which orally administered β-1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. J. Immunol. 173, 797–806.

Johnson, K.J., Glovsk, M., Schrier, D., 1984. Pulmonary granulomatous vasculitis. Pulmonary granulomatous vasculitis induced in rats by treatment with glucan. Am. J. Pathol. 114, 515–516.

Kernodle, D.S., Gates, H., Kaiser, A.B., 1998. Prophylactic anti-infective activity of poly-[1–6]-β-D-glucopyranosyl-[1–3]-β-D-glucopryanose glucan in a guinea pig model of staphylococcal wound infection. Antimicrob. Agents Chemother. 42, 545–549.

Ko, Y.T., Lin, Y.L., 2004. 1,3-β-Glucan quantification by a fluorescence microassay and analysis of its distribution in foods. J. Agric. Food Chem. 52, 3313–3318.

Li, J., Xing, J., Li, D., Wang, X., Zhao, L., Lv, S., Huang, D., 2005. Effects of β-glucan extracted from *Saccharomyces cerevisiae* on humoral and cellular immunity in weaned piglets. Arch. Anim. Nutr. 59, 303–312.

Li, B., Allendorf, D.J., Hansen, R., Marroquin, J., Ding, C., Cramer, D.E., Yan, J., 2006. Yeast beta-glucan amplifies phagocyte killing of iC3b-opsonized tumor cells via complement receptor 3-Syk-phosphatidylinositol 3-kinase pathway. J. Immunol. 177, 1661–1669.

Miura, N.N., Adachi, Y., Yodomae, T., Tamura, S.T., Ohno, N., 2003. Structure and biological activities of β-glucans from yeast and mycelial forms of *Canida albicans*. Microbiol. Immunol. 47, 173–182.

Mueller, A., Raptis, J., Rice, P.J., Kalbfleisch, J.H., Stout, R.D., Ensley, H.E., Browder, W., Williams, D.L., 2000. The influence of glucan polymer structure and solution conformation on binding to (1→3)-β-D-glucan receptors in a human monocyte-like cell line. Glycobiology 10, 339–346.

Nicolosi, R., Bell, S.J., Bistrian, B.R., Greenberg, I., Forse, R.A., Blackburn, G.L., 1999. Plasma lipid changes after supplementation with β-glucan fiber from yeast. Am. J. Clin. Nutr. 70, 208–212.

OECD, 2001. Acute oral toxicity – Fixed dose procedure. In: OECD Guidelines for the Testing of Chemicals. Organization for Economic Co-operation and Development (OECD), Paris, Guideline Test No. 420.

Ohno, N., Suzuki, T., Saito, K., Yadomae, T., 1990. Enhancement of clot formation of human plasma by β-glucans. J. Pharmacobiodyn. 13, 525–532.

Onderdonk, A.B., Cisneros, R.L., Hinkson, P., Ostroff, G., 1992. Anti-infective effect of poly-β1-6-glucotriosyl-β1-3-glucopyranose glucan in vivo. Infect. Immun. 60, 1642–1647.

Patchen, M.L., MacVittie, T.J., 1986. Hemopoietic effects of intravenous soluble glucan administration. J. Immunopharmacol. 8, 407–425.

Riggi, S.J., Di Luzio, N.R., 1961. Identification of a reticuloendothelial stimulating agent in zymosan. Am. J. Physiol. 200, 297–300.

Saitô, H., Yoshioka, Y., Uehara, N., Aketagawa, J., Tanaka, S., Shibata, Y., 1991. Relationship between conformation and biological response for (1→3)-β-D-glucans in the activation of coagulation factor G from limulus amebocyte lysate and host-mediated antitumor activity. Demonstration of single-helix conformation as a stimulant.. Carbohydr. Res. 217, 181–190.

Seljelid, R., Bögwald, J., Lundwall, Å., 1981. Glycan stimulation of macrophages in vitro. Exp. Cell Res. 131, 121–129.

US FDA, 2006. Part 172—Food additives permitted for direct addition to food for human consumption. §172.898—Bakers yeast glycan. In: Code of Federal Regulations (CFR). Title 21—Food and Drugs (US Food and Drug Administration). US Government Printing Office (GPO), Washington, DC , p. 119.

Vetvicka, V., Terayama, K., Mandeville, R., Brousseau, P., Kournikakis, B., Ostroff, G., 2002. Pilot study: orally-administered yeast β1,3-glucan prophylactically protects against anthrax infection and cancer in mice. J. Am. Nutraceut. Assoc. 5, 1–5.

WHO, 1987. Toxicological versus physiological responses. In: Principles for the Safety Assessment of Food Additives and Contaminants in Food. World Health Organization (WHO), International Programme on Chemical Safety (IPCS), Geneva. Environmental Health Criteria, No. 70, p. 82.

Williams, D.L., Sherwood, E.R., Browder, I.W., McNamee, R.B., Jones, E.L., Di Luzio, N.R., 1988. Pre-clinical safety evaluation of soluble glucan. Int. J. Immunopharmacol. 10, 405–414.

Willment, J.A., Gordon, S., Brown, G.D., 2001. Characterization of the human β-glucan receptor and its alternatively spliced isoforms. J. Biol. Chem. 276, 43818–44323.

Xia, Y., Větvička, V., Yan, J., Hanikýřová, M., Mayadas, T., Ross, G.D., 1999. The β-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. J. Immunol. 162, 2281–2290.