Genotoxiciteit van fytosterol(esters)

Genotoxicity of phytosterol(esters)

Aanvulling eerste beoordeling van de veiligheid voor de consument, volgens de Europese verordening 258/97 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten
Addition to the first assessment regarding consumer safety, in accordance with European Regulation 258/97 concerning novel foods and novel food ingredients

Gezondheidsraad:
Commissie Veiligheidsbeoordeling nieuwe voedingsmiddelen (VNV)

Health Council of the Netherlands:
Committee on the Safety assessment of novel foods

aan/to:

de Minister van Volksgezondheid, Welzijn en Sport/
the Minister of Health, Welfare and Sport

de Minister van Landbouw, Natuurbeheer en Visserij/
the Minister for Agriculture, Nature management and Fisheries

Nr 2001/02VNV, Den Haag, 13 december 2001
No. 2001/02VNV, The Hague, December 13, 2001

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Inhoud/Contents

Letter to the Dutch Minister of Health, Welfare and Sport

On December 13, 2001, professor JGAJ Hautvast, Vice-president of the Health Council of the Netherlands wrote as follows to the Minister of Health, Welfare and Sport:

This document has been prepared in response to your request for advice regarding the genotoxicity of sterol metabolites. Phytosterols in use as food ingredients, in particular β-sitosterol, stigmasterol and campesterol, belong to the sterols group. The absorption of cholesterol in the intestine is inhibited by these phytosterols. Cholesterol itself also belongs to the sterols group. The assessment of the safety dossier for the phytosterols was carried out in 1998 by the Dutch Provisional Committee on Safety Assessment of Novel Foods within the context of European Regulation (EC) 258/97. The opinion of the European Scientific Committee on Food was published in April 2000.

At the time of the application for marketing authorisation for phytosterol esters as a food ingredient, the documentation contained findings from *in vitro* genotoxicity studies on phytosterols and phytosterol esters. The genotoxicity studies yielded no evidence of a genotoxic action for phytosterols and their esters. The documentation also contained analytical data on intestinal content in humans, particularly primary and secondary bile acids, sterols and their oxidised and hydrogenated metabolites, in connection with consumption of 8600 mg phytosterols per person per day for 3-4 weeks. The intake recommended by the applicant is 1600 mg per person per day. The daily intake for a consumer with an average dietary pattern is 160-360 mg per person

per day. The change in the quantity of secondary bile acids was studied as these might stimulate the development of intestinal cancer. The change in the quantity of 4-cholesten-3-one was investigated because it had been reported in some scientific publications that 4-cholesten-3-one, a metabolite of cholesterol, had a (geno)toxic effect on murine intestinal cells (Kau87, Suz84, Suz86). In the experimental group with high sterol consumption, the faecal secondary bile acid concentration was significantly reduced at the end of the test period compared with the start, so far as lithocholic acid and cholic acid are concerned. The concentration of lithocholic acid in the experimental group changed from 2.99 $\pm$ 0.28 to 2.26 $\pm$ 0.15 mg per g faeces (dry weight, mean $\pm$ SEM). The concentration of cholic acid changed from 0.18 $\pm$ 0.03 to 0.07 $\pm$ 0.02 mg per g faeces (dry weight). In the experimental group with high sterol consumption, excretion of cholesterol, phytosterols and their metabolites at the end of the test period was significantly elevated relative to the start. Excretion of 4-cholesten-3-one was significant increased in the female volunteers from 0.05 $\pm$ 0.05 to 2.7 $\pm$ 0.58 mg per g faeces (dry weight, mean $\pm$ SEM) and increased in male volunteers from 0.06 $\pm$ 0.04 to 1.1 $\pm$ 0.46 mg per g faeces (dry weight, mean + SEM) (Wes99).

Based on the dossier and the scientific literature, the provisional VNV Committee saw no grounds to ask further questions concerning the genotoxicity of phytosterols.

Over the period that the Human Nutrition Scientific Committee assessed the safety of phytosterol esters, the dossier has been supplemented by the company with genotoxicity research on 4-cholesten-3-one, the aforementioned metabolite of cholesterol. This concerned a bacterial mutagenicity test and an *in vitro* test on chromosomal aberrations in human peripheral blood lymphocytes. The findings from these tests were negative, i.e. no evidence of genotoxicity. An important transformation product of 4-cholesten-3-one, 5β-cholestan-3-one, was also tested in this way and proved likewise non-mutagenic. In addition, two *in-vivo* genotoxicity studies were conducted with phytosterol esters in rats. Excessive DNA reparative synthesis in the liver and the formation of micronuclei in bone marrow served as the indication parameters for genotoxicity. No genotoxic action for the phytosterol esters was found. The Scientific Committee on Food concluded that it had no doubt as to the safety of phytosterol mixtures on the basis of the documentation and the supplements.

In 2000, the Minister for Health, Welfare and Sport asked the Committee on Safety Assessment of Novel Foods of the Health Council of the Netherlands to review all data critically. The Committee commented that a version of the *in-vitro* study on 4-cholesten-3-one was lacking, namely the version in which liver enzymes are used. In

2001, the company supplemented the documentation. The findings of these tests also yield no evidence of a genotoxic action for 4-cholesten-3-one.

The Committee now considers that the applicant has, in a valid and reproducible manner, investigated the genotoxicity of mixtures of phytosterols that are representative of the consumer product and the most important metabolites of cholesterol. The choice of the tests is in accordance with a protocol for genotoxicity testing adopted in the European Union and within OECD member states.

The significance of the exploratory genotoxicity and cytotoxicity research on the metabolite 4-cholesten-3-one described in the scientific literature is considered not to be great by the Committee (Kau87, Suz84, Suz86). The nuclear aberration test (NA) is an unvalidated test that has not been officially acknowledged. The parameters determined indicate a general toxic effect rather than a specific genotoxic effect. This finding can probably be induced with very many substances. The sister chromatid exchange test (SCE) is also not yet internationally accepted. The mechanism is unclear, and the biological relevance is debatable. In addition, 4-cholesten-3-one gives only a marginally positive response.

The Committee considers the genotoxicity tests conducted in accordance with the international protocol to be decisive and regards the phytosterols and cholesterol metabolites tested as non-genotoxic.

I endorse the conclusion of the Committee.

(signed)
professor JGAJ Hautvast

Literatuur/Literature

EG97 Verordening (EG) nr 258/97 van het Europees Parlement en de Raad van 27 januari 1997 betreffende nieuwe voedingsmiddelen en nieuwe voedselingrediënten. Publikatieblad van de Europese Gemeenschappen 1997; L43: 1-6.

EG97a Aanbeveling nr 97/618/EG van de Commissie van 29 juli 1997 betreffende de wetenschappelijke aspecten en de presentatie van de informatie die nodig is om aanvragen voor het in de handel brengen van nieuwe voedingsmiddelen en nieuwe voedselingrediënten te ondersteunen alsmede het opstellen van de verslagen van de eerste beoordeling uit hoofde van Verordening (EG) nr 258/97 van het Europees Parlement en de Raad 1997; L253: 1-36.

FAO96 Biotechnology and Food Safety. Report of a joint FAO/WHO Consultation. Rome, FAO 1996

FAO01 Evalutation of allergenicity of genetically modified foods. Report of a joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology. Rome, FAO 2001.

GR92 Commissie Toxicologische aspecten van biotechnologisch bereide producten. Productveiligheid bij nieuwe biotechnologie. Den Haag, Gezondheidsraad 1992, publicatienummer 1992/03

Kau87 Kaul HK, Gouch DB, Gingerich JD, e.a. Genotoxicity of two fecal steroids in murine colonic epithelium assessed by the sister chromatid exchange technique. Mutagenesis 1987; 2(6): 441-4.

OECD93 Safety evaluation of foods derived by modern biotechnology. Concepts and principles. Paris, OECD 1993.

OECD96 OECD Workshop on Food Safety Evaluation. Paris, OECD 1996.

OECD98 Report of the OECD workshop on the toxicological and nutritional testing of novel foods. Paris, OECD 1998.

OECD00 Report of the task force for the safety of novel foods and feeds. Paris, OECD 2000.

SCF99 Opinion concerning the scientific basis for determining whether food products, derived from genetically modified maize, could be included in a list of food products which do not require labelling because they do not contain (detectable) traces of DNA or protein. Brussels, Scientific Committee on Food of the EU 1999.

SSC99 Opinion of the Scientific Steering Committee on microbial resistance, Brussels, Scientific Steering Committee of the EU 1999.

Suz84 Suzuki K, Bruce WR. Human fecal fractions can produce nuclear damage in the colonic epithelial cells of mice. Mut Res 1984; 141: 35-9.

Suz86 Suzuki K, Bruce WR, Baptista J, e.a. Characterization of cytotoxic steroids in human faeces and their putative role in the etiology of human colonic cancer. Canc Let 1986; 33: 307-16.

Wes99 Weststrate JA, Ayesh R, Bauer-Plank C. Safety evaluation of phytosterol esters. Part 4. Faecal concentrations of bile acids and neutral sterols in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester-enriched margarine. Food chem Tox 1999; 37: 1063-71.

WHO91 Strategies for assessing the safety of foods produced by biotechnology. Report of a joint FAO/WHO Consultation. Geneva, WHO 1991.

WHO00 Safety aspects of genetically modified foods of plant origin. Report of a joint FAO/WHo expert consultation on foods derived from biotechnology. Geneva, WHO 2000.

A	De adviesaanvraag/Request for advice
B	De commissie/The committee
C	EU-procedure/EU-procedure
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E	Aanvullende gegevens/additional data

Bijlagen/Annexes

Bijlage **A**

De adviesaanvraag/Request for advice

Op 18 augustus 1999 schreef de Minister van Volksgezondheid, Welzijn en Sport aan de Voorzitter van de Gezondheidsraad (brief kenmerk GZB/VVB 993428):

Sinds mei 1997 is in de Europese Unie de Verordening (EG) 258/97 van kracht inzake nieuwe voedingsmiddelen en nieuwe voedselingrediënten. Daarmee werd de veiligheidsbeoordeling onderdeel van een communautaire procedure.

Met u is reeds de mogelijkheid besproken de beoordeling door de Gezondheidsraad te laten uitvoeren. Ik verzoek u dan ook mede namens de Staatssecretaris van Landbouw, Natuurbeheer en Visserij, in deze eerste fase van uitvoering van de Europese Verordening (EG) 258/97 gedurende een aantal jaren, de veiligheidsbeoordeling gestalte te geven. Voor het onderbrengen bij de Gezondheidsraad pleit het experimentele karakter dat de beoordeling de eerste jaren zal hebben. Dit experimentele karakter komt voort uit het feit dat het een nieuw soort beoordeling betreft van deels nieuwe categorieën van voedingsmiddelen of voedselingrediënten. Het is namelijk een veiligheidsbeoordeling vóór het op de markt brengen van met name voedingsmiddelen van een genetisch gemodificeerde oorsprong en zogenaamd functional foods (nutriceutica). Daarnaast ga ik ervan uit dat de onafhankelijke wetenschappelijke advisering door de Gezondheidsraad het vertrouwen van de Europese Commissie en de andere lidstaten in het Nederlandse oordeel nog versterkt.

Mijn beleid is erop gericht een zo groot mogelijke openheid en transparantie te realiseren van de gevolgde procedure en de beoordeling om de consument vertrouwen te geven in de veiligheid van de nieuwe

voedingsmiddelen. Ik verzoek de Gezondheidsraad hieraan bij te dragen door bijvoorbeeld inzage te geven in de dossiers waarvoor een aanvraag wordt ingediend, waarbij uiteraard bedrijfsvertrouwelijke gegevens worden beschermd en door de criteria, waarop de veiligheid zal worden beoordeeld, te publiceren.

De Minister van Volksgezondheid, Welzijn en Sport,
(wg) dr E Borst-Eilers

English translation

On 18 August 1999, the Minister of Health, Welfare and Sport wrote as follows to the President of the Health Council (under reference GZB/VVB 993428):

Since May 1977, Regulation (EC) 258/97 concerning novel foods and novel food ingredients has been in force in the European Union. Under the Regulation, the safety of novel foods has to be assessed as part of a community procedure.

Following discussions regarding the possibility of the Health Council making such assessments, the State Secretary for Agriculture, Nature Management and Fisheries and I wish the Council to take reponsibility for safety assessment for a period of several years during the fist phase of implementation of European Regulation (EC) 258/97. It is considered appropriate the Health Council should initially take on this role because the assessment activities will be of an experimental nature, involving both a new form of assessment (i.e. pre-marketing assessment) and, in many cases, new categories of foodstuff (primarily foodstuffs with a genetically modified basis and functional foods or nutraceuticals). We also feel that if assessments are made by a body with the Council's independent scientific status, this will support the validity of the Netherlands'opinion in the eyes of the European Committee and other member states.

My wish is to make the procedure and the assessment as open and transparent as possible, so as to enhance consumer trust in the safety of novel foods. I would like the Health Council to support this objective by, for example, allowing perusal of applicants (insofar as consistent with the need to protect the conficentiality of commercially sensitive information) and publishing the criteria upon which safety assessments are made.

The Minister of Health, Welfare and Sport,
(signed) dr E Borst-Eilers

Bijlage **B**

De commissie/The committee

- Prof. dr LM Schoonhoven, *voorzitter/chairman*
  emeritus hoogleraar entomologie; Wageningen Universiteit en Researchcentrum/ emeritus professor of entomology; Wageningen University and Research centre
- Prof. dr CAFM Bruijnzeel-Koomen
  hoogleraar dermatologie/allergologie; Academisch Ziekenhuis Utrecht/ professor of dermatology/allergology; Academic Hospital Utrecht
- Ir EJ Kok
  toxicoloog; RIKILT-DLO Wageningen/ toxicologist; State Institute for Quality Control of Agricultural Products, Wageningen
- Dr CF van Kreijl
  moleculair-bioloog; RIVM Bilthoven/ molecular biologist; National Institute of Public Health and the Environment, Bilthoven
- Prof. dr P van der Laan
  hoogleraar statistiek; Technische Universiteit Eindhoven/professor of statistics; Technical University Eindhoven
- Dr B Loos, *adviseur/advisor*
  COGEM, Den Haag/Committee on Genetic Modification, The Hague
- Prof. dr FM Nagengast
  gastro-enteroloog; Academisch Ziekenhuis Nijmegen/ gastro enterologist; Academic Hospital Nijmegen

- Dr ir JMA van Raaij
  voedingsfysioloog; Wageningen Universiteit and Researchcentrum/ food physiologist; Wageningen University and Research centre
- Prof. dr G Schaafsma
  hoogleraar voeding; TNO voeding, Zeist/prodessor of nutrition; TNO Nutrition and Food Research, Zeist
- Prof. dr EG Schouten
  hoogleraar epidemiologie; Wageningen Universiteit and Researchcentrum/ professor of epidemiology; Wageningen University and Research centre
- Dr GJA Speijers
  toxicoloog; RIVM Bilthoven/ toxicologist; National Institute of Public Health and the Environment, Bilthoven
- Prof. dr WJ Stiekema
  hoogleraar bioinformatica; Wageningen Universiteit en Researchcentrum/ professor of bioinformatics; Wageningen University and Research centre
- Ir R Top, *adviseur/advisor*
  Ministerie van VWS; Den Haag/ Ministry of Health, Welfare and Sport; The Hague
- Prof. dr WM de Vos
  hoogleraar microbiologie; Wageningen Universiteit en Researchcentrum/ professor of microbiology; Wageningen University and Research centre
- Dr RA Woutersen
  toxicoloog; TNO Voeding, Zeist/toxicologist; TNO Nutrition and Food Research
- Dr JAG van de Wiel, *wetenschappelijk stafmedewerker/scientific staff member*
  Gezondheidsraad, Den Haag/ Health Council of the Netherlands, The Hague

Administratieve ondersteuning: C Nederpelt-Brussee; Gezondheidsraad, Den Haag/ Administrative assistance: C Nederpelt-Brussee; Health Council of the Netherlands, The Hague.

Bijlage **C**

EU-procedure/EU-procedure

Als een fabrikant een nieuw voedingsmiddel op de markt brengt, dient de veiligheid voor de consument gewaarborgd te zijn. In 1997 werd de Europese verordening van kracht waarin de procedure is geregeld voor de goedkeuring voor marktintroductie van een nieuw voedingsmiddel (EG97). Bij deze procedure zijn verschillende actoren betrokken. De aanvrager moet beoordelen of het product werkelijk ‘nieuw’ is, dat wil zeggen dat het nog niet eerder in de Europese Unie in substantiële mate voor menselijke voeding is gebruikt en ook niet wezenlijk gelijkwaardig is aan een bestaand product. (Voor een wezenlijk gelijkwaardig product kan worden volstaan met een kennisgeving van de marktintroductie.) Ook moet het niet gaan om een levensmiddelenadditief, aroma of extractiemiddel, omdat deze producten op een andere wijze worden beoordeeld. Voor een nieuw voedingsmiddel in de zin van de Europese verordening moet de aanvrager een veiligheidsdossier overleggen volgens aanbevelingen van de Europese Commissie (EG97a). Deze aanbevelingen zijn gebaseerd op rapporten van verschillende instanties die zich met het onderwerp nieuwe voedingsmiddelen bezighouden, te weten de OECD (OECD93, OECD96) en de WHO/FAO (WHO91, FAO96). Ook de Gezondheidsraad heeft zich al eerder over dit onderwerp gebogen (GR92). Sinds het verschijnen van de aanbevelingen van de EU wordt in internationaal verband (FAO01, SCF99, SSC99, OECD98, OECD00, WHO00) gewerkt aan explicitering en aanpassing aan de stand van de wetenschap.

De fabrikant levert het volgens de richtlijnen samengestelde dossier in bij het land waar het product het eerst op de markt zal komen. Daarop komt de nationale

veiligheidsbeoordelingsautoriteit in actie. In Nederland is dat de Minister van Volksgezondheid, Welzijn en Sport. Zij heeft de Gezondheidsraad verzocht haar van advies te die-nen. De Voorzitter van de Gezondheidsraad heeft hiertoe de commissie Veiligheidsbeoordeling nieuwe voedingsmiddelen (commissie VNV) ingesteld.

De commissie beoordeelt op basis van de huidige stand van de wetenschap of de door de fabrikant geleverde gegevens juist en volledig zijn en of zij het eens is met diens conclusies. Zij maakt een verslag van haar bevindingen — ook volgens de Europese aanbevelingen (EG97a, deel III) — en biedt dat de minister aan. De minister formuleert het Nederlandse oordeel over een voedingsmiddel en brengt dat in bij het Europese overleg in het Permanent Comité voor levensmiddelen. Alle Europese lidstaten worden uitgenodigd hun oordeel (de zogeheten tweede beoordeling) te geven over het dossier en over de eerste beoordeling alvorens genoemd Comité een eindoordeel velt. Als een dossier veel vragen oproept, gaat er een adviesvraag van de Europese Commissie naar het Wetenschappelijk Comité voor de menselijke voeding. Komt men dan nog niet tot overeenstemming dan beslist de Europese Ministerraad.

English translation

When manufacturers bring novel foodstuffs onto the market, consumer safety has to be assured. In 1997, a European Regulation (EC97) came into force, laying down the procedure for approving the market introduction of novel foodstuffs. The procedure recognises various actors. The applicant must decide whether a product is a novel foodstuff, i.e. a substance that has not previously been available for human consumption to any substantial extent within the European Union and is not substiantially equivalent to any existing product. (If a foodstuff is substiantially equivalent to any existing product, it is sufficient to inform the authorities of its market introduction). Foodstuff additives, aromas and extracts are excluded from the provisions of the directive, since they fall within the scope of an established assessment regime. Before marketing a novel foodstuff, the applicant must compile a safety dossier that complies with the Recommendations of the European Commission (EG97a). These Recommendations are based on reports by a number of bodies that have studied the issue of novel foodstuffs, in particular the OECD (OECD93, OECD96) and the WHO/FAO (WHO91, FAO96). The Health Council of the Netherlands has also considered the question earlier (GR92). Since publication of the EU recommendations, international efforts have been made to clarify and adapt the latest scientific knowledge in the field (FAO01, SSC99, SCF99, OECD98, OECD00, WHO00).
Having compiled a dossier in line with the guidelines, the manufacturer has to submit it to the competent authority in the country where the product is to be marketed first.

This dossier is assessed by the national safety assessment authority. In the Netherlands, this is the Minister of Health, Welfare and Sport, who is advised by the Health Council. The President of the Health Council has created a Committee on the Safety assessment of novel foods (VNV) to advise the minister on behalf of the Council. On the basis of the scientific state of the art, the committee has to decide whether the information provided by the manufacturer is accurate and complete and whether the manufacturer's conclusions are sound. The committee then draws up a report on its findings for the minister; this report must also comply with the European Recommendation (EC97a, part III). After considering the report, the minister formulates the Netherlands'opinion regarding the foodstuff in question, which is discussed at European level in the Standing Committee on Foodstuffs. All other European member states are invited to express a 'second opinion' regarding the dossier and the first opinion. The Standing Committee then arrives at a final judgement. If a dossier is particularly contentious, the European Commission calls upon the Scientific Committee on Food for advice. If consensus still cannot be reached, the issue is referred to the European Council of Ministers.

Bijlage **D**

Overzicht dossiergegevens/ Overview of data in the dossier

Study number: KA960254
Study title: Phytosterols: Bacterial mutation assay
Study results: Two independent mutation tests were performed, in the presence and absence of liver preparations from Arochlor 1254-induced rats. Dose levels of phytosterols (tested in tetrahydrofuran) of 5000, 1500, 500, 150 and 50 µg per plate showed no evidence of mutagenic activity in this bacterial system (TA98, TA100, TA1535, TA1537).

Study number: KA960256
Study title: Phytosterol-esters: Bacterial mutation assay
Study results: Two independent mutation tests were performed, in the presence and absence of liver preparations from Arochlor 1254-induced rats. Dose levels of phytosterol-esters (tested in acetone) of 5000, 1500, 500, 150 and 50 µg per plate showed no evidence of mutagenic activity in this bacterial system (TA98, TA100, TA1535, TA1537).

Study number: KC960255
Study title: Phytosterols: Metaphase chromosome analysis of human lymphocytes cultured *in vitro.*
Study results: Two tests were performed, in the presence and in the absence of liver preparations. Dose levels of phytosterols of 40, 80 and 160 µg per ml culture, after 21

hours of incubation, showed no evidence of clastogenic activity in this *in vitro* cytogenetic test system. Dose levels of phytosterols of 160 μg per ml culture, after 45 hours of incubation, showed no evidence of clastogenic activity in this *in vitro* cytogenetic test system.

Study number: KC960257
Study title: Phytosterol-esters: Metaphase chromosome analysis of human lymphocytes cultured *in vitro.*
Study results: Two tests were performed, in the presence and in the absence of liver preparations. Dose levels of phytosterol-esters of 25, 50 and 100 μg per ml culture, after 21 hours of incubation, showed no evidence of clastogenic activity in this *in vitro* cytogenetic test system. Dose levels of phytosterols of 100 μg per ml culture, after 45 hours of incubation, showed no evidence of clastogenic activity in this *in vitro* cytogenetic test system.

Study number: 97037-E
Study title: Faecal bile acids and sterols in subjects fed controlled diets with or without added vegetable oil sterols.
Study results: Consumption of vegetable oil phytosterols slightly but significantly increased the faecal concentration of 4-cholesten-3-one. This concentration increased in females from 0.05 $\pm$ 0.05 to 2.7 $\pm$ 0.58 mg/g dry weight faeces. In males from 0.06 $\pm$ 0.04 to 1.1 $\pm$ 0.46 mg/g dry weight faeces. These values are still in line with values reported in the literature for subjects fed high or low fat diets. There was no increase in faecal secondary bile acids and sterol oxides could not be detected. The authors of the study mention that all tree compounds are studied in the ongoing scientific research on the causal factors of large bowel cancer.

Bijlage **E**

Aanvullende gegevens/additional data

Study number: KA980008
Study title: Plant sterols: bacterial mutation assay.
Study results: It was concluded that plant sterols (in acetone) did not induce mutation in four strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and one strain of *Escherichia coli* (CM891) under the conditions employed, which included treatments at concentrations up to 5000 μg per plate, in the absence and in the presence of a metabolic activation system (S9).

Study number: KC980009
Study title: Plant sterols: *in vitro* mammalian chromosome aberration test in human lymphocytes.
Study results: Two tests were performed, in the presence and in the absence of liver preparations. Dose levels of phytosterols of 50, 100 and 200 μg/ml culture, after 3 hours of incubation, in the presence and in the absence of liver preparations, showed no evidence of clastogenic activity in this *in vitro* cytogenetic test system. Dose levels of phytosterols of 50, 100 and 200 μg per ml culture, after 21 hours of incubation, in the absence of liver preparations, showed no evidence of clastogenic activity in this *in vitro* cytogenetic test system.

Study number: KA980261
Study title: 4-Cholesten-3-one: reverse mutation in five histidine requiring strains of

Salmonella typhimurium.
Study results: It was concluded that 4-cholesten-3-one (in acetone) did not induce mutation in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) under the conditions employed, which included treatments at concentrations up to 2500 μg per plate (a precipitating dose), in the absence of a metabolic activation system (S9).

Study number: KC980260
Study title: 4-Cholesten-3-one: induction of chromosome aberrations in cultured human peripheral blood lymphocytes.
Study results: Treatment of cultures with 4-cholesten-3-one in the absence of S9 for 3 or 20 hours resulted in frequencies of cells with structural aberrations that were similar to those seen in concurrent solvent control cultures. Frequencies of aberrant cells in the majority of 4-cholen-3-one treated cultures fell within the historical negative control range.

Study number: KA980259
Study title: 5β-Cholestan-3-one: reverse mutation in five histidine-requiring strains of *Salmonella typhimurium.*
Study results: It was concluded that 5β-cholestan-3-one (in acetone) did not induce mutation in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) under the conditions employed, which included treatments at concentrations up to 5000 μg per plate (a precipitating dose), in the absence of a metabolic activation system (S9).

Study number: KC980258
Study title: 5β-Cholestan-3-one: induction of chromosome aberrations in cultured human peripheral blood lymphocytes.
Study results: Treatment of cultures with 5β-Cholestan-3-one in the absence of S9 for 3 or 20 hours resulted in frequencies of cells with structural aberrations that were similar to those seen in concurrent solvent control cultures. Frequencies of aberrant cells in all 5β-Cholestan-3-one treated cultures fell within the historical negative control range.

Study number: KU990111
Study title: Plant sterol ester SSE26698-02: measurement of unscheduled DNA synthesis in rat liver using an *in vivo/in vitro* procedure.
Study results: Treatment with 800 or 2000 mg/kg plant sterol ester did not produce a

group mean NNG (net grain count) value greater than -0.4 nor were any more than 2.7% cells found in repair at either dose. The sterol ester did not induce UDS.

Study number: KC990110
Study title: Plant sterol ester SSE26698-02: induction of micronucleï in the bone marrow of treated rats.
Study results: It is conluded that plant sterol ester SSE26698-02 did not induce micronucleï in the polychromatic erythrocytes of the bone marrow of male rats treated up to 2000 mg/kg/day.

Study number: KA010009
Study title: 4-cholesten-3-one: reverse mutation in five histidine-requiring strains of *Salmonella typhimurium*.
Study results: No evidence of mutagenic activity was obtained with 4-cholesten-3-one in either the presence or absence of metabolic activation in the bacterial mutation assay. Concentrations were up to precipitating test doses (625 and/or 2500 μg per plate)

Study number: KC010008
Study title: 4-cholesten-3-one: induction of chromosome aberrations in cultured human peripheral blood lymphocytes.
Study results: The results of the study indicate that 4-cholesten-3-one showed no evidence of mutagenic activity in vitro, either the presence or absence of metabolic activation in the in vitro chromosome aberration assay in human lmphocytes. Dose levels were up to 1000 μg per ml.