



## SUMMARY & ASSESSMENT

The purpose of this evaluation is to determine whether or not ecdysterone is suitable for use as an ingredient in Class 1 (currently Listed) medicines, and to consider whether any restrictions should be imposed to assure its safety-in-use.

### Characterisation of the substance

Ecdysterone is the most common member of a group of about 300 ecdysteroids, substances that have been isolated from animal (zooecdysteroids) and plant (phytoecdysteroids) sources. They are recognised as acting as steroidal hormones in insects, controlling the various stages of development and metamorphosis, but their function in plants is still conjectural. They are thought to provide some protection against non-adapted phytophagous insects and soil nematodes. Ecdysteroids are structurally quite different from mammalian steroids; they are not expected to bind to vertebrate steroid receptors, and thus are not considered to have hormonal activity in humans. However, this assertion appears to be problematic considering the biological effects, in particular an anabolic one, which were reported in a number of clinical trials (see below).

Ecdysterone itself has been well characterised as a single crystalline chemical entity having a definition in ChemIDplus, a systematic chemical name, a structural chemical formula and a recorded melting point, as outlined in Section 2.

It should be pointed out, however, that the substance actually used as an ingredient in dietary supplements, while being described as ecdysterone (or by one of its synonyms), may not be the pure single substance. This situation arises because other members of the ecdysteroid family are similar in chemical and physical properties, and consequently the naturally occurring mixture of ecdysteroids is difficult to resolve into its individual components. For instance: the research-grade ecdysterone used in one pre-clinical study in 1995 contained 96% ecdysterone (characterised by mass spectrometry) with 4% contamination by other structurally-related ecdysteroids; the commercial product Ecdysone (which was used as the source of ecdysterone in a number of other pre-clinical and clinical trials) was found on assay to contain 4.3 mg of ecdysterone per 5 mg tablet (86%) of 'an ecdysteroid mixture of which ecdysterone was by far the major component'; and the '20-Hydroxy-ecdysterone' (a synonym for ecdysterone) supplied to one sponsor contained only 60% '20-Hydroxy-ecdysterone', while the product supplied to another sponsor was claimed to contain a minimum of 90% 20-hydroxyecdysone (see Attachment 1) .

Thus, the purity of substances being marketed as ecdysterone may be uncertain, and this issue must be managed by attention to Compositional Guidelines, GMP and QC. However, this is not, in itself, a reason for ecdysterone to be excluded from being listed as a Class 1 substance.

### History and pattern of use

A number of medicinal herbs that have been used for several centuries in various herbal traditions, notably in Asia, have recently been found to contain ecdysteroids. These herbs are frequently used as tonics or adaptogens. A list of more than 18 such medicinal herbs is shown in Table 3.1 on page 18; in many of these, ecdysteroids are thought to be active constituents. It is now recognised that ecdysteroids are widely distributed in the plant kingdom. They have been detected in about 6% of the plant species analysed up to the year 2001, which can contain up to 1-2% ecdysteroids calculated on dry plant weight. In typically consumed human foods, however, the ecdysterone content is lower, estimates ranging from 2 µg/kg to 1 g/kg of the food plant. Spinach is often quoted as being comparatively high in ecdysteroids, containing 50 mg/kg of fresh weight. To date, no quantitative epidemiological data have been reported on the estimated human consumption of ecdysteroids in food or herbal medicines.

Much information on the history and pattern of use, and research, on ecdysteroids has been published over the past 40 years, but mostly in the Russian, Japanese and Chinese languages. This evaluation has relied largely upon the limited information available in the English abstracts of some of the original publications, and on several apparently reliable English language expert reviews.

The pattern of use of ecdysterone in dietary supplement preparations has included the treatment of athletes for bodybuilding, muscle strengthening and endurance enhancement as well as the management of clinically diagnosed diseases. The doses used for both athletic and medicinal purposes in the literature surveyed were similar, in the range 10-30 mg/day (including use in children treated for diarrhoea), for periods of up to two months but usually less. One exception was a clinical trial in which ecdysterone was used at 5 mg/kg of body weight daily for ten days, or 300 mg/day for a 60-kg person.

The dose recommended by the NZ sponsor who notified Medsafe of specific formulations was 200 mg/day 'ecdysterone' (in two capsules twice a day) for six weeks, which is a higher daily dose than most doses reported in clinical trials in the literature, although it should be noted that the NZ supplements were based on a product assayed at only 60% ecdysterone (thus the actual dose of ecdysterone would be 120 mg/day).

It may also be noted that the pre-clinical trials reviewed employed doses of at least 2.5 mg/kg/day (rats, for 8 weeks), frequently 5 mg/kg/day (mice and rats, for 7-15 days), and in one trial 100 mg/kg/day (quail, for 4 weeks). This would equate to a range of 150-6000 mg/day for a 60-kg person.

However, as indicated below, there are major differences in pharmacokinetics of the substance between humans and experimental animals, with the latter showing a much shorter elimination half-life compared with the former.

### **Biological activity**

Reviewed literature suggests that ecdysterone may have a number of therapeutically relevant biological activities. These potential effects include, but may not be limited to: anabolic; immunostimulant; stimulation of carbohydrate metabolism; normalisation of lipid biosynthesis and hepatoprotection; normalisation of renal function; antiarrhythmic action and cardiovascular protection; and antioxidant effects.

It needs to be stressed that the efficacy of ecdysterone was not evaluated as part of this report, and that biological activity data have been reviewed in terms of possible toxicological implications, such as the claimed anabolic activity.

The evidence for many of these activities is difficult to evaluate because as indicated in the main body of the report, a number of references were published in languages other than English. The majority of the evidence to support above-listed effects has been obtained in animal models, with some immunomodulating effects demonstrated *in vitro*. The most extensive evidence obtained over the last 40 years supports the claims of anabolic activity. This and the *in vitro* immunostimulant effects also support the traditional use of ecdysterone-containing herbs as tonics and adaptogens. Some immunological trials have produced conflicting results, with either no effect demonstrated or, in one case, the inhibition of production of antibody cells by spleen in mice.

Research since about 1990, on the possibility of influencing or inducing human gene expression using ecdysteroids, has resulted in the development of a licensed system known as the Ecdysteroid-Inducible Mammalian Expression System, which is used for research purposes only. Such inducible expression systems are already showing potential for clinical use in human gene therapy. Applications for regulated gene expression include inducible gene targeting, normalising the overexpression of toxic and teratogenic genes, blocking of

antisense RNA expression, studying development and other physiological processes, and gene therapy. Examples of the last include the controlled expression of anti-inflammatory cytokines to treat autoimmune diseases and graft-versus-host pathology, or the regulated production of recombinant insulin to treat Type 1 diabetes (Graham 2002, cited by Bathori & Pongracz 2005). This research raises the question of safety-in-use in terms of unwanted or unforeseen pharmacological effects arising from the therapeutic use of ecdysterone as an ingredient in Class 1 medicines, and warrants a brief discussion here.

In insects, pulses of ecdysteroids including ecdysterone during late larval and prepupal development coordinate the activation of a large number of primary and secondary response genes, signalling the onset of metamorphosis. Ecdysteroids act as the key signals that trigger the major postembryonic transitions during the life cycle of the insect. Ecdysteroid pulses induce transcription factors that, in turn, coordinate the expression of downstream target genes. These changes in gene expression direct the animal through the remarkable developmental changes associated with metamorphosis. As steroids, ecdysteroids pass through the cell membrane into the cell nucleus. There, they bind to ecdysteroid receptor (EcR) proteins. The ecdysteroid/EcR complex then combines with a third protein, the ultraspiracle receptor for ecdysteroid (UsP). The ecdysteroid/EcR/UsP complex triggers ecdysteroid response elements on the genome and thereby promotes gene transcription; the first genes to be activated are called early response genes. This is a code for proteins that are themselves gene regulatory factors, either alone or in combination with hormones. They activate late-response genes that code for the proteins that actually cause structural changes, cell differentiation, or apoptosis (programmed cell death). The active EcR/UsP heterodimer in insects is structurally akin to the vertebrate RXR heterodimeric class (that includes the nonsteroidal thyroid, retinoic acid and vitamin D receptors), rather than to the vertebrate homodimeric steroid receptors such as those for glucocorticoid, mineralocorticoid, progesterone and androgens. However, EcR is the only characterised non-mammalian nuclear hormone receptor with no apparent mammalian homologues.

Therefore, ecdysteroids are said to be unlikely to exert any hormonal effect on mammalian cells in the absence of pre-treatment according to the Ecdysteroid-Inducible Mammalian Expression System. Furthermore, ecdysterone is not listed among the most potent ecdysteroids screened for use in this approach to gene therapy.

Nevertheless, anabolic effects observed in a range of reported clinical trials suggest that ecdysterone may act via another, as yet undetermined, mechanism on the cell's metabolism, producing effects similar to those mediated by human steroid hormone receptors.

### **Toxicology**

Ecdysterone is consistently described in the literature as having very low, or no, oral toxicity in humans (although clinical evidence in support of such assertions is lacking). Yet, as mentioned above, given at about 300 mg/day, the substance has been shown to induce anabolic-like effects in athletes (see below and section 6).

In an attempt to establish the LD<sub>50</sub> in mice, oral administration at the maximum practicable dose, 9.0 g/kg, did not produce any deaths. Therefore, a murine oral LD<sub>50</sub> was not established.

An attempt to establish the repeat-dose toxicity of an ecdysteroid mixture containing 50% ecdysterone was similarly unsuccessful, with male and female rats fed 2000 mg/kg/day of the mixture for 35 days showing no toxicity. This dose contained 1000 mg/kg/day ecdysterone, which would equate to 60g/day for a 60-kg person, a factor of 300 times the comparatively high dose recommended by one of the NZ sponsors.

No information was found on the genotoxicity of ecdysterone *in vivo*. One trial *in vitro* with human leucocytes found no significant effect on the chromosomal structure.

Carcinogenic potential of ecdysterone appears to not have been investigated, but two trials testing antitumour potential of the compound showed no potentiation of tumorigenesis in selected animal tumour models, although the stimulation of the growth of mammary gland carcinomas was observed in one investigation in mice.

One study relevant to the potential reproductive effects of oral doses of ecdysterone found no significant effect on the weight of ventral prostate or seminal vesicles in either adult or immature male rats dosed orally at 5 mg/kg/day for 10 days. In contrast, intraperitoneal injections of ecdysterone in male mice at doses of 4-100 mg/kg/day for 30 days were found to inhibit the growth of seminal vesicles (by about 53%) and decrease sperm production (by about 26%). The IP injections of ecdysterone in female mice caused variations in the length of the reproductive cycle, and reduced the number of *corpora lutea* developing in each ovary. The relevance of these findings to oral doses of ecdysterone in humans is unclear, especially as pharmacokinetic studies of ecdysterone suggest that the metabolic fate of ecdysteroids depends greatly on the route of administration (intraperitoneal and subcutaneous compared with oral), and the metabolism of ecdysteroids is significantly different in different mammalian species. For example, the elimination half-time in man was about 9 h, thus being considerably longer than about 0.4 h measured in an animal model (sheep).

It is therefore possible that the virtual lack of toxicity in animals, particularly rodents, may not be suggestive of a low risk of human toxicity because of apparent species-specific differences in metabolism of ecdysterone.

This clearly suggests that toxicological profile of ecdysterone has not been adequately investigated to assure its safety-in-use, particularly the long-term safety.

### **Clinical data**

Although a number of clinical trials of ecdysterone and other ecdysteroids have been carried out, their usefulness to this evaluation of toxicity and safety-in-use is limited by the fact that the reports were published in languages other than English, by their short duration, and the typical lack of reporting of either presence or absence of adverse effects. The clinical trials summarised in Table 1 on page 7 (only abstracts and reviews were available in English) did not mention any toxic or adverse effects, although a reasonably wide range of populations (including children, patients and athletes) were treated for various periods from five days to two months. The dose of ecdysterone used varied up to about 300 mg/day; this may be compared with that recommended by one NZ sponsor (200 mg/day). Importantly, these clinical trials can not be accepted as adequate evidence of safety-in-use because of the range of methodological limitations listed above.

### **Adverse reactions**

Neither the Australian Adverse Drug Reactions Advisory Committee nor the New Zealand Centre for Adverse Reactions Monitoring has received any reports of adverse reactions to preparations or products containing ecdysterone. No data were retrieved from the WHO on adverse reactions relating to ecdysterone.

### **Conclusion**

The available pre-clinical and clinical evidence suggests that the potential for oral toxicity of ecdysterone in humans is low, but the strength of the evidence is limited by the relative paucity of well designed toxicological studies, the apparent pharmacokinetic differences between humans and test animals, and the lack of long-term clinical safety studies.

Assertions in the literature that ecdysterone is unlikely to have a hormonal action in humans may be problematic in the light of anabolic-like effects observed in clinical studies on athletes. If this activity, as yet unexplained, is hormonal, it may be a possible indicator of unwanted, and therefore, potentially-serious adverse effects. On the other hand, available data appear to suggest an array of beneficial pharmacological effects (e.g. hypoglycaemic, hypocholesterolaemic, anabolic, antiasthenic), thus, providing a possible explanation of the properties of several plant species widely used in traditional medicine, which are known to contain plant sterols.

The observations of reproductive toxicity and carcinogenicity caused by intraperitoneal and subcutaneous administration in rodents are of uncertain relevance to human oral use, because of wide differences in pharmacokinetics observed between species and administration routes. Thus, it is not possible to assess the long term safety-in-use of ecdysterone because of the lack of formally and robustly designed scientific studies.

Nevertheless, it may be noted that research over the last 40 years has revealed the presence of ecdysteroids, mainly ecdysterone, in several human food plants (albeit at comparatively low concentrations). Among the human food plants, spinach is reported to have an unusually high ecdysterone content as mentioned above; thus the consumption of 150-600 g/day of spinach would contain an amount of ecdysterone equivalent to the doses used in some of the clinical trials summarised in Table 1 below. Ecdysterone has also been found in many traditional medicinal herbs that have been used for several centuries, although it is unclear what level of exposure to the substance was attained by such use. However, no toxic effects ascribable to ecdysterone have been reported in that context.

**Table 1 Summary of selected clinical trials of ecdysterone**

<b>Study design (Reference)</b>	<b>Subject details</b>	<b>Treatment details (dose and route)</b>	<b>Endpoints</b>	<b>Key outcomes</b>	<b>ADRs</b>
20 days Not controlled Saydakhmedova & Kuznetsova (1999)*	n =137 children Diarrhoea patients	15-30 mg/d oral (compared with Retabolil 1 mg/d)	Body weight and biochemical assay	Body weight normalised, without virilisation or inhibition of enterocytic enzymes.	None reported
30 days Not controlled Syrov et al. (2004)*	n =not stated in abstract Hepatitis B patients	5 mg BD oral plus conventional therapy	Clinical and biochemical assay	Improved clinical and biochemistry, normalised autoimmunity, compared with conventional therapy alone.	None reported
10 days Not controlled Osipova et al. (2002)*	n =35 Giardiasis patients	5 mg TID or QID oral, then higher dose in 4 patients.	Clinical and microbiological assay	Clearance in 25/35 pts. Increased by 3/4 on higher dose.	None reported
Review of 10 clinical studies* 5 days – 2 months Bathori & Pongracz (2005)	n >417 total Athletes and patients, aged 18-61 years	Various oral, usually 7.5-15 mg/day	Various	Various positive outcomes	None reported

\*Abstracts only. Original clinical reports available only in Russian.

## Table of contents

<b>1</b>	<b>INTRODUCTION .....</b>	<b>10</b>
<b>2</b>	<b>CHARACTERISATION OF THE SUBSTANCE.....</b>	<b>10</b>
2.1	General Information.....	10
2.1.1	Names and synonyms.....	10
2.1.2	Approved names.....	11
2.2	Descriptions.....	11
2.2.1	Physicochemical details.....	11
2.3	Manufacture.....	11
2.3.1	Manufacturer(s).....	11
2.3.2	Description of manufacturing process and process controls.....	11
2.3.2.1	Control of Materials.....	13
2.3.2.2	Control of critical steps and intermediates.....	13
2.3.2.3	Process validation and/or evaluation.....	13
2.3.2.4	Manufacturing process development.....	13
2.4	Characterisation.....	13
2.4.1	Monographs.....	13
2.4.2	Phytochemical characterization.....	13
2.4.3	Impurities and contaminants.....	13
2.5	Control of the Active Substance.....	14
2.5.1	Specifications.....	14
2.5.1.1	Draft compositional guideline.....	14
2.5.2	Analytical procedures.....	15
2.5.2.1	Validation of analytical procedures.....	15
2.5.2.2	Batch analyses.....	15
2.5.2.3	Justification of specification.....	15
2.5.3	Reference standards or materials.....	15
2.5.4	Container closure system.....	15
2.5.5	Stability.....	15
<b>3</b>	<b>HISTORY AND PATTERN OF USE.....</b>	<b>16</b>
3.1	Traditional Uses.....	16
3.1.1	Dietary.....	16
3.1.2	Therapeutic.....	16
3.2	Existing Availability and Regulatory Status.....	16
3.2.1	Products manufactured and/or sold in NZ in 2003.....	16
3.2.2	Products available in Australia.....	16
3.2.3	Products available internationally.....	16
3.3	Posology.....	17
3.3.1	Route of administration.....	17
3.3.2	Dose form.....	17
3.3.3	Dosing range, frequency and duration.....	18
3.3.4	Indications and contraindications.....	18
<b>4</b>	<b>BIOLOGICAL ACTIVITY.....</b>	<b>18</b>
4.1	Primary Pharmacodynamics.....	18
4.1.1	In vitro.....	18
4.1.1.1	Anabolic effect.....	18
4.1.1.2	Immunostimulant effect.....	18
4.1.2	In vivo.....	19
4.1.2.1	Anabolic effect.....	19
4.1.2.2	Stimulation of carbohydrate metabolism.....	19
4.1.2.3	Effect on lipid biosynthesis and hepatoprotection.....	19
4.1.2.4	Correction of experimental renal dysfunction.....	20

4.1.2.5	Antiarrhythmic and other cardiovascular effects .....	20
4.1.2.6	Antioxidant effect .....	20
4.1.2.7	Effects on tumours .....	20
4.2	Secondary Pharmacodynamics .....	21
4.3	Safety Pharmacology .....	21
4.3.1	Gene expression modifying effects .....	21
4.3.2	Effect on tumour growth in rodents .....	21
4.3.3	Immunomodulating effects .....	21
4.3.4	Endocrinological effects .....	21
4.3.5	Pharmacodynamic drug interactions .....	21
4.4	Pharmacokinetics .....	22
4.4.1	Absorption and distribution .....	22
4.4.2	Metabolism and excretion .....	23
4.4.3	Pharmacokinetic drug interactions .....	23
<b>5</b>	<b>TOXICOLOGY .....</b>	<b>23</b>
5.1	In vitro .....	23
5.2	Single-dose Toxicity .....	24
5.2.1	Mouse .....	24
5.2.2	Rabbit .....	24
5.3	Repeat-dose Toxicity .....	24
5.4	Genotoxicity .....	24
5.4.1	In vitro .....	24
5.4.2	In vivo .....	24
5.5	Carcinogenicity .....	24
5.6	Reproductive and Developmental Toxicity .....	25
5.6.1	Fertility .....	25
5.6.2	Embryonic and foetal development .....	25
5.6.3	Prenatal and postnatal development .....	25
5.7	Local Tolerance .....	26
<b>6</b>	<b>CLINICAL DATA .....</b>	<b>26</b>
6.1	Treatment of Diarrhoea .....	26
6.2	Treatment of Hepatitis B .....	26
6.3	Treatment of Giardiasis .....	26
6.4	Review of Clinical Trials of Ecdysterone .....	26
<b>7</b>	<b>ADVERSE REACTIONS .....</b>	<b>27</b>
7.1	Australian Adverse Reactions Database .....	27
7.2	New Zealand Adverse Reactions Database .....	27
7.3	International Adverse Reaction Databases .....	28
7.4	Literature-reported Events .....	28
<b>8</b>	<b>REFERENCES .....</b>	<b>29</b>



used the names '20-Hydroxy-ecdysterone' and 'hydroxy-ecdysterone', which are not supported by rigorous scientific literature. There are 13 other synonyms (see below).

### 2.1.2 Approved names

'Ecdysterone' is not an Australian Approved Name (AAN), but subject to consideration by the appropriate joint Agency naming committee in a due course, it is expected to be approved as an AAN (or an equivalent).

## 2.2 Descriptions

### 2.2.1 Physicochemical details

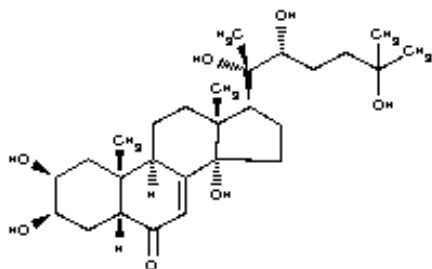
Information extracted from ChemIDplus:

MeSH Heading: Ecdysterone

Systematic name: 5- $\beta$ -Cholest-7-en-6-one, 2- $\beta$ ,3- $\beta$ ,14,20,22,25-hexahydroxy-, (22R)-.

CAS Registry Number: 5289-74-7

Chemical structure of ecdysterone:



Molecular formula:  $C_{27}H_{44}O_7$

Ecdysterone is a crystalline compound melting at 240-242°C (Merck Index). Matsuda et al. (1970) give the melting point of ecdysterone as 242°C (decomp).

(The latter authors also give values for molecular weight, UV spectrographic data and optical rotation.)

## 2.3 Manufacture

### 2.3.1 Manufacturer(s)

One sponsor provided a certificate of analysis from their suppliers, the Rocky Mountain Chemical Company (USA) (see Attachment 1). It was not clear whether this company was a manufacturer or simply the importer/exporter of the substance.

### 2.3.2 Description of manufacturing process and process controls

No information has been provided by sponsors, other than the very limited outline of the extraction of ecdysterone from *Cyanotis vega* given by one sponsor.

The research papers and reviews consulted for this evaluation indicated that ecdysterone may be extracted from a number of plants. Bathori & Pongracz (2005) give an account of the manufacture of commercial quantities of ecdysterone from *Silene italica* ssp *nemoralis*, Bandara et al. (1989) mention the yield of ecdysterone (3.2%) obtained from the mature stem of *Diplocisia glaucescens* (= *Cocculus macrocarpus*), while Slama & Lafont (1995) describe

Ecdisten (commercial ecdysterone) as being derived from the roots of *Leuzea carthamoides* (= *Rhaponticum carthamoides*). The last authors also mention the seeds of *L. carthamoides* as containing 2-3% ecdysterone, and a tea containing 0.08-0.22% ecdysterone made from the dried green parts of the same herb. The ecdysterone used by XXXXXXXXXXXXXXXXXXXX was stated on their Certificate of Analysis to be derived from Vega Root Extract (see Attachment 1, Certificate of Analysis).

The manufacturing steps of ecdysterone are as described by Bathori & Pongracz (2005). The isolation procedure is given by the authors for purification of ecdysteroids from *Silene italica ssp nemoralis*. However, the same or a very similar isolation cascade may be generally used to prepare ecdysteroids from other plant sources.

“The scheme for producing ecdysterone, and subsequently other ecdysteroids, involves three main steps:

extraction and effective pre-purification of the crude extract;

separation of ecdysteroids using preparative scale chromatography; the initial step is adsorption, followed by efficient and selective reversed-phase liquid chromatography using low-pressure operation; and

purification is sometimes completed by the use of HPLC and preparative TLC.

The exhaustive extraction of ecdysteroids from the dried plant requires a relatively large volume of a polar solvent, such as methanol. The solvent-plant ratio may be 10:1. The extraction results in an 8-fold purification. For pre-purification we have used a charged-based batch separation, which has an important role in the effectiveness of the first step preparative scale purification of the ecdysteroids. The pre-purification also involves fractionated precipitation, solvent-solvent distribution and a special hybrid chromatographic method .

The fractionated precipitation is done repeatedly (three times); the methanolic solution is mixed with half the amount, with the same volume, and with the doubled volume of acetone. After each precipitation step, the solution consists of ecdysteroids, but the precipitate is also washed with the same ratio of methanol and acetone. The washing solution is also combined with the solution. After combination, the solutions are evaporated, dissolved in methanol, and subjected to acetone precipitation.

After acetone precipitation, the solution is taken to dryness, and dissolved in 50% aqueous methanol. The solvent-solvent distribution is done between aqueous methanol and hexane; the ecdysteroids are in the aqueous methanol (bottom) phase. After separating the phases, the bottom phase is evaporated, and the residue is dissolved in plain methanol.

The methanolic solution is mixed with aluminium oxide, and the suspension is taken to dryness in a rotatory evaporator.

The sample-stationary phase ratio is small (1:2), and results in the removal of a number of contaminants and in the enrichment of the ecdysteroids. Elution is carried out with different volume ratios of dichloromethane and ethanol (96%), such as 95:5, 9:1, 8:2 and 1:1. The ecdysteroids of our interest were eluted with dichloromethane-ethanol (96%) (9:1 and 8:2). This special hybrid chromatographic method possesses some characteristics of solid-phase extraction, representing an efficient, simple, specific and economic purification with high sample load for group separation. The separation is based on adsorption/desorption processes on alumina. Because of the strong interaction between the matrix components and the adsorbent, an abundant amount of contaminant is retarded on the adsorbent (aluminium oxide) and the ecdysteroids are selectively eluted with the proper mobile phase(s). The adsorption of certain ecdysteroids to the adsorbent is reduced using a water-containing eluent. In spite of the adsorption the single employment of aluminium oxide stationary phase is necessary at least once, because of the potency of extremely high sample load (e.g. 200 g

sample on 400 g of aluminium oxide). This special liquid chromatographic method uses a gradient elution in column chromatography but only with four gradient steps. These provide coarse separation according to the adsorption ability of the ecdysteroids.”

[The above pre-purification procedure yields a liquid phase containing principally ecdysterone, which is purified by crystallisation. The remaining mother liquor may then be further processed to give other ecdysteroids.]

#### **2.3.2.1 Control of Materials**

No information available (not critical for an active ingredient of Listable/Class 1 medicines).

#### **2.3.2.2 Control of critical steps and intermediates**

No controls specified (not critical for an active ingredient of Listable/Class 1 medicines).

#### **2.3.2.3 Process validation and/or evaluation**

No information available (not critical for an active ingredient of Listable/Class 1 medicines).

#### **2.3.2.4 Manufacturing process development**

No information available (not critical for an active ingredient of Listable/Class 1 medicines).

### **2.4 Characterisation**

#### **2.4.1 Monographs**

No internationally-recognised monographs were available. Three reviews of ecdysteroids in general provided some useful information for this evaluation (Bathori & Pongracz 2005; Lafont & Dinan 2003; Slama & Lafont 1995).

#### **2.4.2 Phytochemical characterization**

No information on phytochemical characterization of ecdysterone was provided by the sponsors. Bathori & Pongracz (2005) have published HPLC curves for an extract containing ecdysterone, and for ecdysterone prepared and purified by preparative normal-phase (NP) and reversed-phase (RP) preparative HPLC.

#### **2.4.3 Impurities and contaminants**

Ecdysterone is manufactured by solvent extraction from plant material followed by physicochemical methods of separation, and purification by crystallisation. The commercial products described as ecdysterone (or its synonyms) are therefore likely to contain compounds, in addition to ecdysterone, that have similar solubilities and chromatographic parameters. This issue has received very little attention in the literature.

For instance, the commercial product Ecdysten (Ecdisten) advertised as containing 5 mg of 20-hydroxyecdysone per pill was found on analysis to contain 4.3 mg/pill of ecdysteroids of which 20-hydroxyecdysone (ecdysterone) was by far the major component (Slama & Lafont 1995). Another product described as 20-hydroxyecdysterone (see Attachment 1) has a specification minimum assay of 95% (presumably ecdysteroids) but a 20-hydroxyecdysterone content of only 60%.

The certificate of analysis provided by one sponsor included specifications for heavy metals (<10 ppm calculated as Pb) and arsenic (<3 ppm), and for microbiological contamination (see Attachment 1).

## 2.5 Control of the Active Substance

The certificate of analysis provided by one sponsor (see Attachment 1) showed specifications including a minimum assay of 95% (presumably of ecdysteroids, but not stated) and 60% of '20-hydroxy-ecdysterone' (ecdysterone). The supplier's data sheet provided by another sponsor (see Attachment 1) claimed a 20-hydroxyecdysone content of 90%.

### 2.5.1 Specifications

Specifications were included in the certificate of analysis provided by the USA supplier to one NZ sponsor, reproduced in Attachment 1. These specifications are shown in Table 2.1. One other sponsor provided a very brief specification (see Attachment 1).

**Table 2.1 Specifications for ecdysterone (Rocky Mountain Chemical Company)**

Test	Specifications
Loss on drying	1.0% (sic) <sup>a</sup>
Solution (appearance)	Brown
Residue on ignition	>5% (sic) <sup>b</sup>
Heavy metals (as Pb)	<10 ppm
Assay	min 95%
Arsenic	<3 ppm
Botanical source	Vega root
Active constituent	20-Hydroxy-ecdysterone
Microbiological	Standard Plate Count max 3000/g
	Yeast/mould max 50/g
	Coliform negative/g
	<i>E. coli</i> negative/g
	<i>Salmonella</i> negative/g
	<i>Listeria</i> negative/g

a - presumably 'NMT 1.0%' is intended.

b - presumably 'NMT 5.0%' is intended.

#### 2.5.1.1 Draft compositional guideline

Compositional Guidelines (CG) for ecdysterone have not been developed by any of the sponsors, but a draft CG including information from the sponsor's specifications (see Attachment 1) is suggested below.

The specifications shown in Table 2.2 do not address phytochemical profile (i.e. markers) that need to be included in the CG in order to control adequately the quality and safety of the herb-derived material. Subject to the approval of ecdysterone for use in Class 1 medicines, the CG will need to be developed further and finalised after consultation with industry.

**Table 2.2 Draft Compositional Guideline for ecdysterone**

<b>Test</b>	<b>Requirements and Limits</b>	<b>Test Method/Additional Requirements</b>
Appearance	Crystalline powder	Visual
Loss on drying	NMT 1%	As per Appendix IX D of the <i>BP</i>
Assay	Requirements to be determined	HPLC or another validated analytical method.
Pesticide Residues	Complies with limits	As per Appendix XI L of the <i>BP</i> .
Residue on ignition	NMT 5% (need to conduct tests to determine typical values)	As per Appendix XI J of the <i>BP</i>
Microbiological	As dictated in the <i>ARGCM</i> Part III and/or Joint Agency guidelines	According to the <i>BP</i> test limits
Heavy metals (total)	As per the <i>ARGCM</i> Part III and/or Joint Agency guidelines	According to the <i>BP</i> test limits
Arsenic	As per the <i>ARGCM</i> Part III and/or Joint Agency guidelines	According to the <i>BP</i> test limits
Solvent residues	Complies with limits for specific solvents in the IHC topic Q3C	According to the <i>BP</i> test limits

**2.5.2 Analytical procedures**

No analytical procedures were specified by the sponsors.

**2.5.2.1 Validation of analytical procedures**

No information provided.

**2.5.2.2 Batch analyses**

The Certificate of Analysis of one batch of ecdysterone was provided by one sponsor (see Attachment 1).

**2.5.2.3 Justification of specification**

No justification has been provided for the specifications.

**2.5.3 Reference standards or materials**

As mentioned above, one of the sponsors provided in-house specifications for ecdysterone from one US manufacturer (Rocky Mountain Chemical Company).

**2.5.4 Container closure system**

No information available (not critical for a raw ingredient of Listable/Class 1 medicines).

**2.5.5 Stability**

No information available (not critical for a raw ingredient of Listable/Class 1 medicines).

The dates of manufacture and expiry shown on the Certificate of Analysis (see Attachment 1) appeared to indicate an expected stability of four years for the certified batch of ecdysterone.

### **3 HISTORY AND PATTERN OF USE**

#### **3.1 Traditional Uses**

##### **3.1.1 Dietary**

Phytoecdysteroids are found in many plant species where they can reach concentrations above 1-2% of the plant dry weight (Lafont & Dinan 2003). While many of these plants have been used extensively in traditional herbal medical systems in Asia, the normal human diet contains much smaller amounts of ecdysteroids. The ecdysteroid content of generally-consumed human foods is variously estimated at from about 2 µg/kg to 10-1000 mg/kg of food; for instance it is particularly high in spinach (50 mg/kg of fresh weight), white beet and quinoa seed (Bathori & Pongracz 2005; Slama & Lafont 1995).

No estimation of typical dietary intake of ecdysterone, or of phytoecdysteroids, was available, but of all typically eaten vegetables the spinach has the highest content of ecdysterone (50 mg/kg fresh weight). Consequently, a usual portion (100-200 g) of fresh spinach would be expected to deliver 5-10 mg of ecdysterone. Contributions of other foods to the daily intake of ecdysterone are likely to be minimal.

##### **3.1.2 Therapeutic**

A number of medicinal herbs that have been used for a long time in various herbal traditions, (such as those of India and China), have been found to contain ecdysteroids. These are frequently used as tonics or adaptogens. A selection is shown in Table 3.1 below; biological effects considered to be due (at least in part) to ecdysteroids are marked with an asterisk (Bathori & Pongraz 2005; Slama & Lafont 1995).

#### **3.2 Existing Availability and Regulatory Status**

##### **3.2.1 Products manufactured and/or sold in NZ in 2003**

Two products containing ecdysterone were notified to Medsafe as having been sold in NZ in 2003. These are:

NFS Ecdy-Meth 600 (contains 50 mg ecdysterone per capsule); and

NFS Sterone (contains 50 mg ecdysterone per capsule).

Both were sold by one sponsor, XXXXXXXXXXXXXXXXXXXXXXXX who suggested a dose of two capsules twice a day for six weeks.

##### **3.2.2 Products available in Australia**

There are no ARTG entries for products containing ecdysterone as an ingredient. But as indicated above, a large number of herbs and/or herb-derived ingredients are expected to contain this substance as an obligatory component.

##### **3.2.3 Products available internationally**

Klein (2004) stated that more than 140 dietary supplements containing ecdysteroids including ecdysterone were available on the Internet and elsewhere, such as: Syntrolol, Ecdysten, Ecdybol and MethoxyFactor (all of which are shown as registered trade names). Slama & Lafont (1995) also mentioned Maralan, a green tea preparation available in the Czech Republic, and Leuzea Drops, an alcoholic concentrate that (according to the authors' HPLC analysis) contained relatively high concentrations of ecdysteroids, also available in the Czech or Slovak Republics.

**Table 3.1 Ecdysteroid-containing medicinal herbs used in traditional medicine**

Plant species	Effects (therapeutic use)	Reference
<i>Achyranthes radix</i>	Diuretic, tonic*	Hikino & Takemoto (1972)
<i>Ajuga iva</i>	Antidiabetic*	Wessneretal. (1992)
<i>Asparagus dumosus</i>	Diuretic, antiseptic, anthelmintic*, astringent, sexual enhancer, refrigerant	Bathori & Pongraz (2005)
<i>Boerhaavia diffusa</i>	Diuretic	Surietal. (1982)
<i>Cenipodium album</i>	Anthelmintic*	Bathori & Pongraz (2005)
<i>Cyanotis arachnoida</i>	Skin disease*	Bathori & Pongraz (2005)
<i>Cyathula capitata</i>	Diuretic, tonic*	Hikino & Takemoto (1972)
<i>Diploclisia glaucescens</i>	Biliousness, venereal diseases, spermicidal*	Miller etal. (1985)
<i>Helleborus sp.</i>	Stomachic, bitter tonic*, analgesic*, antirheumatic	Colombo et al. (1990)
<i>Ipomoea calonyction</i>	Febrifuge, purgative	Canonica et al. (1975)
<i>Leuzea carthamoides</i>	Tonic*, roborant*, adaptogenic*, antidepressive*	Syrov & Kurmukov (1977)
<i>Paris polyphylla</i>	Hypotensive	Singh&Thakur(1982)
<i>Pfaffia iresinoides</i>	General stimulant*, 'Brasil ginseng'*	Nishimotoetal. (1988)
<i>Polypodium decumanum</i>	Tonic*, antiinflammatory*, antioxidant*, neuroprotective*	Bathori & Pongraz (2005)
<i>Sida carpinifolia</i>	Stomachic, tonic, antipyretic*, anti-inflammatory, antidepressive*	Pandit etal. (1976)
<i>Taxus baccata</i>	Anticancer	Bathori & Pongraz 2005
<i>Taxus cuspidata</i>	Antidiabetic	Nakano et al. (1982)
<i>Vitex glabrata</i>	Astringent,antihelminthic*, gastro-intestinal disorders, vulnerary*, sexual enhancer*	Werawattanametin et al. (1986)

\* indicates that ecdysteroids are considered to be the active principles.

### 3.3 Posology

#### 3.3.1 Route of administration

The products notified by NZ sponsors are intended for oral use only.

#### 3.3.2 Dose form

The substances notified by NZ sponsors are in capsule form.

### **3.3.3 Dosing range, frequency and duration**

Slama & Lafont (1995) indicated that the recommended dose of Ecdisten (Ecdysten) was 5-10 mg (1-2 tablets) orally three times a day before meals. This would amount to 15-30 mg/day of the commercial product consisting principally of ecdysterone. The recommended course of treatment with Ecdysten was 15-20 days, which could be repeated if necessary in 1-2 weeks. No reasons were given for the limited length of the course, and no limitations on the number of repeats were stated.

XXXXXXXXXXXXXXXXXXXXX informed Medsafe that their NFS Ecdy-Meth 600 and NFS Sterone were usually used at a dose of four capsules/day (containing 200 mg/day ecdysterone) for six-week periods.

### **3.3.4 Indications and contraindications**

Among the indications for Ecdysten, Slama & Lafont (1995) listed asthenic and astheno-depressive states, weakening of the organism, long (alcohol) intoxications, somatic and infectious diseases, neurasthenia, neurosis, hypotension, and mental and physical fatigue. However, no information was provided by the sponsors concerning indications or contraindications. The customary use in NZ appears to be by bodybuilders and sportspeople.

## **4 BIOLOGICAL ACTIVITY**

In the most up-to-date and comprehensive survey of ecdysterone and related compounds reviewed for this evaluation, *Phytoecdysteroids - from isolation to their effects on humans* (Bathori & Pongraz 2005), a large number of pharmacological effects were described with references to original papers. For most of the original papers relevant to this evaluation, only the abstracts were available in English, the main articles being in Russian, Chinese or Japanese.

Ecdysteroids have a hormone function in insects and crustaceans. However, their human and animal pharmacological effects are not considered to be hormonal activity (Bathori & Pongraz 2005).

See section 4.2 below and the Summary and Conclusion section (page 4) for comments on the potential of ecdysterone for interaction with human genes.

The biological activities that may be most relevant to human toxicity or safety-in-use are exemplified by reference to selected animal studies below, with as much experimental detail as was available in the abstracts.

### **4.1 Primary Pharmacodynamics**

#### **4.1.1 *In vitro***

##### **4.1.1.1 Anabolic effect**

Syrov (1984) suggested that the anabolic effect observed in white mice (see 4.1.2.1 below) was connected with a rise in polyisome functional activity and an increase in the rate of protein macromolecule formation rather than an induction of RNA synthesis, on the basis of studies on murine liver tissue *in vitro*.

##### **4.1.1.2 Immunostimulant effect**

Fomovska et al. (1992) (cited by Bathori & Pongracz 2005) used an *in vitro* model of lymphocytes to test the effects of ecdysteroids including ecdysterone. The ecdysteroids

stimulated DNA synthesis in the lymphocytes, activated by concanavalin A (ConA), in the concentration range  $10^{-12}$  to  $10^{-5}$  mol/L. At concentrations above  $10^{-4}$  mol/L, however, the ecdysteroids including ecdysterone inhibited the activating effect of the mitogen. Similarly, Chiang et al. (1992) (also cited by Bathori & Pongracz 2005) reported that  $2 \times 10^{-9}$  mol/L of ecdysterone stimulated the DNA synthesis in splenocytes activated by ConA.

#### **4.1.2 *In vivo***

##### **4.1.2.1 Anabolic effect**

Syrov (1984) showed that ecdysterone (and other ecdysteroids) at an oral dose of 5 mg/kg/day (duration of trial not stated in abstract) in white mice stimulated protein synthesis, increasing body mass, muscle mass and muscle strength. No untoward effects on the mice were noted.

Slama et al. (1995) extracted ecdysterone in 96% purity from seeds of *Leuzea carthamoides* (which contain 2% ecdysteroids). Feeding the ecdysterone at a dose of 100 mg/kg/day to Japanese quail (N=10) over a period of four weeks led to a 15% greater increase in body weight compared with controls (N=10), significant at  $P < 0.001$ .

##### **4.1.2.2 Stimulation of carbohydrate metabolism**

Yoshida et al. (1971) studied the effects of ecdysterone on rats and mice with high blood glucose levels induced by administration of glucagon, alloxan, or anti-insulin serum. Ecdysterone was injected IP at a dose of 0.5 mg/kg bw (mice weighing 20 g, rats 200 g). The blood glucose level was approximately halved by the ecdysterone treatment in alloxan-induced diabetic mice, while the ecdysterone treatment had no effect on the blood glucose levels of control (healthy) mice. Changes in the specific activities of some enzymes related to glucose metabolism seemed to be responsible for promoting glucose utilisation in the hyperglycaemic animals (Uchiyama & Yoshida 1974).

Similarly, Syrov et al. (1992b) reported a 'considerable decrease' in the free fatty acids in the blood serum of rats (body weight 180-220 g) dosed orally with 5 mg/kg/day of ecdysterone for 15 days. The free fatty acids had been 'sharply increased' by the prior injection of alloxan (150 mg/kg). Other parameters including glycogen, malonic dialdehyde, pyruvic acid and Ca-transporting fraction of the liver mitochondria were also 'normalised'. The mechanism was thought to include the normalisation of the phospholipid content of the liver mitochondrial membranes, which had been reduced by alloxan-induced insulin insufficiency.

##### **4.1.2.3 Effect on lipid biosynthesis and hepatoprotection**

Mironova et al. (1982) treated experimental hypercholesterolaemic rats with 2.5 mg/kg/day (presumably oral, but not stated) of ecdysterone. After 3, 6 and 8 weeks the cholesterol in blood plasma was reduced by 7.0%, 16.9% and 29.0% respectively. This effect was accompanied by a decrease in the cholesterol content of erythrocyte membranes as well as microfilaments of the erythrocyte border, by 26% and 34% respectively. The amount of phospholipids and the ratio of cholesterol to phospholipids were also normalised in the membranes. The authors concluded that there was competition between cholesterol and the hypercholesterolaemic drug (ecdysterone) during binding with the membrane sites, depending on their concentrations in plasma, intestinal lumen and membranes.

Syrov et al. (1986) studied the effect of 5 mg/kg/day oral doses of ecdysterone for 7 days in normal healthy rats. The chemical composition of the bile was significantly modified ('improved' according to the authors) by increased levels of bile acids and bilirubin and by decreased cholesterol content. Of a number of ecdysteroids tested, ecdysterone exerted the

most beneficial effect on the parameters studied in rats with toxic hepatitis induced by heliotrine.

#### **4.1.2.4 Correction of experimental renal dysfunction**

A hypoazotaemic effect in male rats with experimentally-induced renal dysfunction was observed by Saatov et al. (1999) (cited by Bathori & Pongracz 2005). Rats weighing 120-150 g were dosed intragastrically with 5 mg/kg of ecdysterone. The urea content of the blood was determined two hours after the first dose, while residual nitrogen was determined after repeated daily doses for 10 days. The ecdysterone treatment caused significant decreases in both urea content and residual nitrogen content of the blood.

#### **4.1.2.5 Antiarrhythmic and other cardiovascular effects**

Kurmukov & Ermishina (1991) (cited by Bathori & Pongracz 2005) reported an 'intense effect' (presumably beneficial) of intraperitoneal doses in the range 5-20 µg/kg on artificially-induced cardiac arrhythmia in rats. Further, the 20 µg/kg dose increased the survival rate by 75% in cases of arrhythmia induced by either aconite or CaCl<sub>2</sub>.

Khushbaktova et al. (1991) (cited by Bathori & Pongracz 2005) reported that, in experimentally-induced atherosclerosis in rabbits, the oral administration of 10 mg/kg/day for 28 days restored the normal level of microsomal Na/K ATPase enzyme activity, decreasing the enzyme activity by nearly 60%.

#### **4.1.2.6 Antioxidant effect**

Several authors have described antioxidant effects of ecdysterone (Bathori & Pongracz (2005) cited and summarised six original research papers). The antioxidant activity of ecdysterone was as high as that of known inhibitors of lipid peroxidation, such as diethyl para-phenylenedione and ethylenediaminetetraacetate and was comparable with that of hydroquinone.

#### **4.1.2.7 Effects on tumours**

Hirono et al. (1969) inoculated (IP) 11 male rats (Moriyamaso MP-1 strain) with 10<sup>7</sup> tumour cells of Yoshida ascites sarcoma. Three experimental groups were formed: four animals were injected with ecdysterone (10 mg/kg IP daily for three days beginning on the third day after inoculation), three animals were injected IP with inokosterone (similar protocol) and the remaining four animals were given IP injections without the ecdysteroids as controls. The mitotic index (number of mitoses/2000 tumour cells) was calculated with Giemsa-stained smear preparations of the tumour ascites withdrawn just before, and at intervals up to 24 hours after, the first injection. There was no statistically significant difference between the control and the ecdysteroid injected groups ( $P > 0.05$ ). Also, there were no statistically significant differences between the control and the ecdysteroid injected groups concerning the subsequent invasive growth of tumours and the animals' life span.

Lagova & Valueva (1981 abstract only) investigated the effects of ecdysterone on transplantable tumours in mice and rats. Doses of ecdysterone in the range 0.1-300 mg/kg administered SC daily for five days showed no antitumour activity against leukaemia (L1210 and La) and some types of solid tumours of mice (Lewis lung carcinoma LLC, and cancer of the cervix RShM-5). However, in contrast to the above study, ecdysterone stimulated the growth of mammary gland carcinomas (Ca-755 and RMK-1) in mice and rats.

[No further information available from abstract; article in Russian]

## **4.2 Secondary Pharmacodynamics**

No specific data available, but see above.

## **4.3 Safety Pharmacology**

### **4.3.1 Gene expression modifying effects**

Research since about 1990 has studied the possibility of influencing or inducing human gene expression using ecdysteroids. The perceived possibility of using ecdysterone in human gene therapy therefore raises the question of unwanted or unforeseen pharmacological effects arising from the therapeutic use of ecdysterone, and warrants brief consideration here. The research has found that ecdysteroids operate in non-mammalian cells via specific hormone receptor proteins, ecdysteroid receptors (EcR), which have been found to be absent in mammalian cells. Thus, for gene expression to be influenced in mammalian cells by ecdysteroids, the EcR must first be introduced into the target cells. In the absence of the EcR, ecdysteroids are unlikely to exert any hormonal effect on mammalian cells. (Bathori & Pongracz 2005; Graham 2002, cited by Bathori & Pongracz 2005). This issue is discussed further in the Summary and Conclusion section (page 4 above).

### **4.3.2 Effect on tumour growth in rodents**

In the work of Lagova & Valueva (1981) mentioned in subsection 4.1.2.7 above, ecdysterone stimulated the growth of mammary gland carcinomas of mice and rats (Ca-755 and RMK-1). [No other information was available from the abstract of the Russian article.]

### **4.3.3 Immunomodulating effects**

Kuzmitsky et al. (1990) showed that the commercial drug Ecdysten (which is principally ecdysterone) in single doses in the range 5-20 mg/kg administered into the gut of mice stimulated the primary immune response slightly as measured by the indices of T-cell activity and phagocyte functions. On the other hand, a higher dose of 50 mg/kg (route not stated) inhibited the number of antibody cells in the mouse spleen. No other information was available from the abstract of the Russian article.

In the *in vitro* experiments of Sakhibov et al. (1989) with cultures of human lymphocytes, following their polyclonal stimulation with phytohaemagglutinin, no immunomodulatory effect of ecdysteroids (10<sup>-7</sup>-10<sup>-5</sup> mol/L) on lymphocyte transformation could be demonstrated (cited by Slama & Lafont 1995).

### **4.3.4 Endocrinological effects**

Slama & Lafont (1995) reviewed the publications of a number of workers who sought evidence of any androgenic, gonadotropic and thymolytic activity of ecdysterone. Sergeev et al. (1991) investigated the thymolytic activity of anabolic steroids in mice (weight 18-20 g) after administration of 50 µg/kg daily (route not stated) for 10 days. Under these conditions testosterone and metandrostenalone exhibited clear thymolytic effects, whereas ecdysterone was ineffective. In other work with ecdysterone or Ecdysten, these substances were not associated with any of the adverse androgenic, antigonadotropic or thymolytic side effects shown by the vertebrate anabolic steroid hormones (Kuzmitsky et al. (1990), Sergeev et al. (1991), Syrov (1984); cited by Slama & Lafont 1995).

### **4.3.5 Pharmacodynamic drug interactions**

Konovalova et al. (2002) found that ecdysterone significantly stimulated the chemotherapeutic effect of reduced doses of the cytostatic drugs cisplatin and adriamycin on

SC and IP transplanted P38 and L1210 leukaemia and metastasizing B16 melanoma in mice. Six to eight BDF1 hybrid mice weighing 21-24 g were used for each experiment. Cisplatin was given on days 2, 4, 6 and 8, either alone (2 or 4 mg/kg IP) or with ecdysterone (2 mg/kg cisplatin and 10 mg/kg ecdysterone). Adriamycin was given on days 1 and 6, either alone (3 or 6 mg/kg) or with ecdysterone (3 mg/kg adriamycin on days 1 and 6, with 50 mg/kg ecdysterone on days 1, 2, 6 and 7). A dose of 50 mg/kg ecdysterone alone on days 1, 2, 6 and 7 was also tested.

The combination of the low dose of the cytostatic agents with ecdysterone (10 mg/kg and 50 mg/kg respectively) gave better or superior results in all parameters tested (inhibition of tumour growth, mouse survival rate, lifespan, and antimetastatic activity) compared with the high dose of the cytostatic agents alone. Ecdysterone alone increased the lifespan by 18%, while in combination with adriamycin, the mean increase in the lifespan was >140%.

#### **4.4 Pharmacokinetics**

##### **4.4.1 Absorption and distribution**

Hikino et al. (1972 a; abstract only; article in Japanese) studied the pharmacokinetics of <sup>3</sup>H-ecdysterone in mice. After oral administration of 35 mg/kg ecdysterone, organs, tissues and fluids were harvested at time intervals ranging from 10 minutes to 72 hours for radioactivity assay by scintillation counting. After 72 hours, less than 0.5% of the administered radioactive dose remained in the body (mainly in liver and blood).

The same authors (Hikino et al. 1972 b), using autoradiographic techniques, observed that the absorption of <sup>3</sup>H-ecdysterone from the gastrointestinal tract in mice was slow and limited following oral administration of 35 mg/kg compared with its absorption from the peritoneum. Radioactivity was retained in the gastrointestinal tract, migrating from the stomach to the intestine over a period of eight hours, at which time (the last autoradiogram illustrated in the article) high radioactivity still remained in the gastrointestinal contents while elimination occurred via biliary excretion. The gall bladder radioactivity reached a maximum level about one hour after administration and maintained a high level for at least four hours post dose (the time of the last gall bladder autoradiogram illustrated in the article). Most of the radioactivity in the body had disappeared in less than 24 hours.

In a review of the metabolic fate and pharmacokinetics of ecdysteroids, Simon & Koolman (1989) concluded that vertebrates were unable to break down the steroid skeleton of the molecule (a situation analogous to that of vertebrate-type steroids, which are converted by enzymatic oxidation, reduction and conjugate formation to compounds that are eventually excreted via urine and faeces). Simon (1988) found that ecdysteroids given orally to mammals pass the acidic conditions of the stomach without apparent changes in their structure. Experiments with sheep and man showed that after oral dosing ecdysteroids were absorbed and reached a peak blood concentration within 30 minutes to two hours after administration (Simon (1988), cited by Simon & Koolman 1989). Small concentrations of ecdysteroids could be detected in saliva and larger concentrations in urine, with concentrations in these fluids closely following the blood levels. However, the recovery of unchanged ecdysteroids in saliva and urine was low, and represented only a few percent of the oral dose. (The faecal route of elimination is much more important, as indicated below. The bioavailability of ecdysterone is not known.)

Slama et al. (1995) fed Japanese quail with 0-500 mg/kg ecdysterone (purified to 96%), and with *Leuzea carthamoides* seeds (containing 2% ecdysteroids estimated to contain 100-180 mg/kg of ecdysterone equivalent) daily for four weeks, measuring the increase in body mass obtained with the ecdysterone feed compared with controls. Ecdysterone at

100 mg/kg gave 15% increase, seeds 9.5% increase. It was reported that the concentration of ecdysterone in the birds' serum increased uniformly with the amount of ecdysterone in the feed, with the maximum of about 30 ng/mL found in animals given the feed containing 500 mg/kg ecdysterone.

#### 4.4.2 Metabolism and excretion

In the pharmacokinetic study by Hikino et al. (1972 a) mentioned above, following the oral administration to mice of 35 mg/kg <sup>3</sup>H-ecdysterone, a total of 74.2% of the administered radioactive dose was recovered in the urine and faeces (10.5% and 63.7%, respectively). This contrasted with a total of 95.9% of the administered radioactive dose being recovered in the urine and faeces after intraperitoneal dosing (4.7% and 91.2% respectively).

The same authors (Hikino et al. 1972 b) concluded that the elimination of the orally administered ecdysterone appeared to be fast, and its excretion pattern confirmed that faecal excretion (mostly derived from biliary excretion) was much more important than urinary excretion. Most of the radioactivity in the body had disappeared in less than 24 hours.

Dzhukharova et al. (1987) also showed that ecdysteroids are rapidly eliminated from the blood. After oral application to man the elimination half-time ( $t_{1/2}$ ) was 9 h. In sheep, the  $t_{1/2}$  of ecdysterone was 0.4, 0.2 and 2.0 h after oral, IV and IM administration, respectively. These lower figures indicated a faster rate of elimination in sheep (lamb) compared with man (adult).

In mice, after injection (route not stated) of ecdysterone the elimination  $t_{1/2}$  was 9 min (Dzhukharova et al. (1987), cited by Simon & Koolman 1989).

Lafont et al. (1988) found that about 55% of ecdysteroids were metabolised following injection (IP) of labelled ecdysone (not ecdysterone) into mice. The metabolism of ecdysteroids in vertebrates was considered to be simple in contrast to the diverse reaction products detected in invertebrates; only a few metabolites were detected in mice. At least some of these were thought to be formed by intestinal flora and parasites in the animal; no polar metabolites or conjugates were detected.

Tsitsimpikou et al. (2001) studied the excretion of ecdysterone in human urine after a single oral dose of 20 mg of ecdysterone (in 4 x 5 mg tablets of Ecdysten) in a single volunteer. Using gas chromatography (GC) coupled with mass spectrometry (MS) and high-resolution mass spectrometry (HRMS) they detected two metabolites, and the parent ecdysterone, in the urine up to 68 hours after administration. The main metabolite was identified as deoxyecdysone (amount not stated), detected up to 21 hours post-administration. A second metabolite, 2-deoxyecdysterone, and unchanged ecdysterone were present at lower levels accounting for 0.15% and 0.19% of the administered dose, respectively.

#### 4.4.3 Pharmacokinetic drug interactions

No information available.

## 5 TOXICOLOGY

### 5.1 *In vitro*

Bandara et al. (1989) reported that ecdysterone showed no activity as a molluscicide (against *Biomphalaria glabrata* snails), no antifungal activity (against *Cladosporium cladosporioides*), no antibacterial activity (against *Mycobacterium fortuitum*, *Eschericia coli* and *Staphylococcus aureus*) and no anti-inflammatory activity (leukotrien, LTB<sub>4</sub>, assay).

## **5.2 Single-dose Toxicity**

### **5.2.1 Mouse**

The LD<sub>50</sub> (oral, mouse) of ecdysterone is reported as >9.0 g/kg, with oral administration at the maximum practicable dose of 9.0 g/kg producing no fatalities (Ogawa et al. 1974).

### **5.2.2 Rabbit**

A 0.1 g/kg intravenous dose of ecdysterone administered to rabbits was not followed by any toxic reaction (Kholodova 2001, cited by Bathori & Pongracz 2005).

## **5.3 Repeat-dose Toxicity**

Matsuda et al. (1970) (cited by Ogawa et al. 1974) fed rats with a mixture of half ecdysterone and half inokosterone to investigate the sub-acute toxicity of phytoecdysteroids. Male and female rats (number not stated in citation) received 200-2000 mg/kg/day of the mixture orally for 35 days. During this period, body weight and feed consumption were recorded, and at the end of the testing period selected haematological and clinical chemistry parameters (erythrocytes, haemoglobin, protein, cholesterol and transaminase activity), bromosulphalein-excretion, and the weights of various organs (pituitary, thyroid, thymus, lung, heart, liver, spleen, kidney, adrenal, prostate, epididymis, seminal vesicle, uterus and ovary) were determined. There were no significant differences for the investigated parameters between animals on the test diet and the controls, and no toxicity occurred (Matsuda et al. 1970, article in Japanese; cited by Ogawa et al. 1974, and by Simon & Koolman 1989).

## **5.4 Genotoxicity**

### **5.4.1 *In vitro***

In a chromosome aberration test (pilot study), Burdette (1974 b) cultured human leucocytes from four healthy individuals (three male, one female) in Waymouth's medium with 20% foetal-calf serum, heparin and phytohemagglutinin at 37°C in the presence of ecdysterone (final medium concentrations of 5 ng/mL - 55 µg/mL) for the last 24 h of the 96 h culture time. For negative control, leucocytes were cultured without the exposure to ecdysterone; positive control conditions were not described.

After washing, the cells were plated, fixed and stained (with Giemsa and/or quinacrine mustard) on glass slides, and the number of chromosomes was determined photo microscopically for a total of 485 preparations (366 exposed to ecdysterone and 119 without ecdysterone). The morphology of the treated chromosomes was similar to that of the controls, except for the number of cultures exhibiting 60 or more chromosomes (hyperploidy).

Leucocytes exposed to ecdysterone (5 ng/mL - 55 µg/mL) showed a slight, non-significant and not dose-dependent increase (8%) in the incidence of hyperploidy (29/366) compared with the negative controls (5%; 6/119).

### **5.4.2 *In vivo***

No data available.

## **5.5 Carcinogenicity**

No data available.

## 5.6 Reproductive and Developmental Toxicity

Stopka et al. (1999) tested the effects of intraperitoneal injections of ecdysterone on growth and reproduction of juvenile and adult mice of both sexes. The injections corresponded to final doses in the range 4-100 mg/kg daily for 30 days. The effects on growth were evaluated by monitoring of the daily bodyweight gains. In addition, the effects on female reproduction were determined by vaginal smears, while the effects on male reproduction were evaluated by size of seminal vesicles and by the content of sperm in the caudal epididymis. It was found that ecdysterone enhanced growth in the groups of female juveniles but not in the male juveniles. In the adults, ecdysterone appeared to cause small increases in growth in both males and females, although only in the males ( $n=5$  or  $6$ , dose =  $1$  mg/day, or about  $40$  mg/kg/day) was the increase (7.6%) statistically significant ( $P<0.0001$ ); the growth effect in females was confounded by normal reproductive cyclical changes. Similarly, only in adult males was an increase in liver weight (9.8%) statistically significant ( $P$  not stated).

In contrast to these anabolic effects, intraperitoneal ecdysterone inhibited growth of seminal vesicles and decreased sperm production. The sperm count was reduced in the adult mice in a dose-dependent manner, by about 25.6% (from  $7.4 \times 10^9$  to  $5.5 \times 10^9$  per mL,  $P<0.0001$ ) at the highest dose of 40 mg/kg daily IP. The seminal vesicle weight was reduced by about 53% (from 0.46 g to 0.25 g, corrected for body weight,  $P<0.0001$ ) by the same dose, although dose dependency was not apparent. In the adult females the possibility of hormonal interaction of ecdysterone was unclear but could not be precluded. Although the lengths of the various stages of the sexual cycle observed (prooestrus, oestrus, metoestrus and dioestrus) were not changed, the overall length of the cycle was increased in a non-dose-dependent manner from about 4.3 days to about 7.5 days during the 30-day trial. After termination of the trial, the ovaries were examined for the presence of *corpora lutea*. The females receiving IP ecdysterone (all doses) had only 2-3 *corpora lutea* in each ovary while the controls showed the presence of multiple *corpora lutea* (number not stated). The authors likened these effects to the hyperoestrogenic syndrome that occurs in domestic animals after consuming phytoestrogen-containing food.

### 5.6.1 Fertility

Prabhu & Nayar (1974) investigated possible sex hormone activity of ecdysterone in 4-day cycling adult female mature white rats. Ecdysterone was administered intravaginally (30-500  $\mu$ g/animal, single dose). The effects were examined by vaginal smears and compared with the effects of administration of 1  $\mu$ g oestradiol dipropionate. No oestrogenic or anti-oestrogenic effects of ecdysterone were observed (cited by Slama & Lafont 1995).

Bandara et al. (1989) reported that, in *in vitro* tests designed to identify potential chemical spermicides, ecdysterone at a concentration of 20 mg/mL caused 100% immotility of human spermatozoa in fresh human semen within 20 seconds.

Syrov (2000) observed no significant effects on the weight of the ventral prostate or seminal vesicles in either adult male or immature (including castrated) male rats, in contrast to the significant anabolic effects in heart and skeletal muscle in adult mature and immature rats (and kidney in immature rats) observed during the same study. The animals were dosed orally with ecdysterone (among other phytoecdysteroids) at 5 mg/kg/day for 10 days.

### 5.6.2 Embryonic and foetal development

No information available.

### 5.6.3 Prenatal and postnatal development

No information available.

## 5.7 Local Tolerance

No information available.

## 6 CLINICAL DATA

### 6.1 Treatment of Diarrhoea

Saydakhmedova & Kuznetsova (1999) reported on the use of Ecdysten (mainly ecdysterone) (15-30 mg/day for 20 days) in a randomised clinical trial for the correction of the nutritional status of 137 children with persistent diarrhoea syndrome. They were compared with 136 similar children treated with retabolil (1 mg/day for 10-12 days). (According to ChemIDplus, retabolil, or nandrolone decanoate, is an androgenic metabolic steroid used for treating nutritional inadequacy arising from catabolic illness.) Before the trial the patients' decreased nutritive status was reported to be due to intolerance of lactose and gluten. The outcome of the trial was that normal body weight was achieved in the Ecdysten treatment group more rapidly than in the retabolil treatment group, without virilisation or inhibition of enterocytic enzymes. No adverse events were reported (abstract only; article in Russian).

### 6.2 Treatment of Hepatitis B

Following an *in vivo* trial with rats with heliotropine-induced hepatitis, Syrov et al. (2004) included Ecdysten in a complex therapy scheme for human patients with chronic viral hepatitis B (one 5-mg tablet twice a day for 30 days). The inclusion of Ecdysten was reported to substantially improve the clinical and biochemical indices of the functional state of the liver, positively influence the humoral and cell immunity and the resistance factors, and normalise the course of autoimmune processes accompanying the liver pathology. No adverse events were reported arising from the use of Ecdysten (abstract only; article in Russian).

### 6.3 Treatment of Giardiasis

Osipova et al. (2002) reported on the use of Ecdysten (mainly ecdysterone) in the treatment of giardiasis in 32 patients with persistent and 3 patients with acute disease. A ten-day course of Ecdysten (one 5-mg tablet 3 or 4 times a day) resulted in clinical and parasitological recovery in 22/32 (68.7%) and 3/3 patients respectively. Four of the remaining ten non-cured patients were then further treated with an increased dose (not specified), and the recovery was achieved in three of the four patients, increasing the recovery rate to a total of 28/35 (80%) No adverse events were reported (abstract only; article in Russian).

### 6.4 Review of Clinical Trials of Ecdysterone

Bathori & Pongracz (2005) reviewed in English ten clinical trials of ecdysterone, but commented that the original reports were not available to them and so they summarised the results without further details or discussion. Seven of these reports were apparently directed towards confirming the efficacy of ecdysterone in improving the physical ability and performance of athletes, while three reports referred to the treatment of patients with diagnosed clinical conditions. The findings of these trials are summarised below.

Portugalov et al. (1996) reported on a trial of three ecdysteroid-containing preparations on the protein balance and working capacity of body builders and other special interest groups. The anabolic activity and increased working capacity were confirmed.

Simakin (1998) reported on the effect of ecdysterone on muscle tissue mass and fat mass and hormonal changes of 78 highly trained male and female athletes. Over a period of 10 days (presumably including training) the ecdysterone-consuming group (given 5 mg/kg body

weight ecdysterone orally) showed about 7% increase in lean muscle and a 10% reduction of fat tissue. The hormone balance did not change. The effects of ecdysteroids were also tested on 117 highly trained speed skaters between the ages of 18 and 28; work capacity, body weight, lung capacity and VO<sub>2</sub> max were monitored.

Fadeev (no date indicated) performed a study on 112 athletes, 89% of whom were supplemented with ecdysterone versus placebo. The ecdysterone-consuming group showed less fatigue, greater performance, more motivation, greater speed and improvement in strength within five days.

Gadzhieva et al. (1995) reported on the effect of three ecdysteroid-containing preparations including Ecdysten on a sub-population taking daily physical exercise. All three preparations reduced the fat content under daily aerobic training. Further, two preparations including Ecdysten elevated the muscle mass.

Azizov (1997) compared a number of newly-developed ecdysteroid-containing preparations. Treatment of 'high class athletes' for 20 days with Leveton (containing ecdysterone, dose not stated) caused a marked reduction in coagulation potential and an increase in working capacity and rehabilitation ability.

Azizov (1998) reported on the daily administration of 400 mg of Leveton (containing ecdysterone, amount not stated) for 20 days to athletes. Reduction of chemiluminescence of the urine, reduction of malonic dialdehyde, and increase in physical working capacity (bicycle ergometry) were recorded.

Kibrik & Reshetnyak (1996) tested the effect of Leveton (containing 2.5 mg ecdysterone per tablet) on 93 patients (73 men, 20 women) with sexual dysfunction. At a dose of Leveton containing 7.5-10 mg/day ecdysterone for one month followed by a 15-day break and a further month's Leveton therapy, 75% of the patients showed improvements in libido and sexual activity.

Mirzaev (2000) studied the effect of doses of 5 mg and 10 mg ecdysterone daily for 10 days on men with infertility diagnosis (decreased spermatogenesis as a complication of certain urological diseases). Both the copulative function and sperm quality were improved.

Saatov et al. (1999) reported on the effects of ecdysteroids on the microcirculation of patients with chronic glomerulonephritis. A total of 35 patients aged 35±7.1 years (20 female, 15 male) participated in the trial. All patients received the traditional treatment, with 17 receiving an additional 15 mg/day Ecdysten (5 mg TID) for 10 days. The main attention was focused on the study of the morphometric indices of microcirculation, which were improved (especially in the nephrotic form of the disease).

No toxicity or adverse effects were mentioned in any of the above 10 clinical trials reviewed by Bathori & Pongracz.

## **7 ADVERSE REACTIONS**

### **7.1 Australian Adverse Reactions Database**

The Australian Adverse Drug Reactions Advisory Committee has received no reports of adverse reactions to ecdysterone, 20-hydroxyecdysone, Ecdysten, Ecdisen or Leveton.

### **7.2 New Zealand Adverse Reactions Database**

The New Zealand Centre for Adverse Reactions Monitoring (CARM) has received no reports of adverse reactions to ecdysterone, 20-hydroxyecdysone, Ecdysten, Ecdisen or Leveton.

### **7.3 International Adverse Reaction Databases**

No data were retrieved from the WHO on adverse reactions relating to ecdysterone, 20-hydroxyecdysone, Ecdysten, Ecdisen or Leveton.

### **7.4 Literature-reported Events**

No reports of adverse reaction events involving ecdysterone, 20-hydroxyecdysone, Ecdysten, Ecdisen or Leveton treatments were found in the literature.

## 8 REFERENCES

- Azizov AP, Seifulla RD, Ankudinova IA, Kondrateva II and Borisova IG. (1998). *Eksp. Klin. Farmakol.*; 61(1):60. (Article not seen; cited by Bathori & Pongracz 2005.)
- Azizov AP. (1997). *Eksp. Klin. Farmakol.*; 60(5):58. (Article not seen; cited by Bathori & Pongracz 2005.)
- Bandara BMR, Jayasinghe L, Karunaratne V, Wannigama GP, Bokel M, Kraus W and Sotheeswaran S. (1989). Ecdysterone from stem of *Diploclisia glaucescens*. *Phytochemistry*; 28:1073-5.
- Bathori M & Pongracz Z. (2005). Phytoecdysteroids - from isolation to their effects on humans. *Current Medicinal Chemistry*; 12(2):153-72.
- Burdette WJ. (1974). Numbers of chromosomes in human leucocytes exposed to ecdysterone. In: *Invertebrate endocrinology and hormonal heterophyly* (Ed. Burdette WJ). Springer Verlag; 371-4.
- Chiang HC, Wang JJ and Wu RT. (1992). Immunomodulating effects of the hydrolysis products of formosamin C and  $\beta$ -ecdysone from *Paris formosana* Hayata. *Anticancer Research*; 12(5):1475-8.
- Dinan L. (2001). Phytoecdysteroids: biological aspects. *Phytochemistry*; 57:325-39.
- Dzhukharova MK, Sakhibov AD, Kasymov B, Syrov VN, Takanaev AA and Saatov Z. (1987). Experimental Study Of The Pharmacokinetics Of Ecdysterone. *Khimiko-Farmatsevticheskii Zhurnal*; 21(10):1163-7. (Abstract only; article in Russian.)
- Fadeev BC. (No date). Comments on the Results of Retibol in the Practice of Athletic Training and Rehabilitation. Natural Sports Research Institute. URL (accessed November 2005):  
<http://www.bodybuilding.com.store/ecdy.html>
- Fomovska CN, Berdyshev AG and Kholodova Y. *Ukr Biokhim Zh.*; 64(2):56. (Article not seen; cited by Bathori & Pongracz 2005.)
- Gadzhieva RM., Portugalov SN, Paniushkin VV and Kondrateva II. (1995). *Eksp. Klin. Farmakol.*; 58(5):46. (Article not seen; cited by Bathori & Pongracz 2005.)
- Hikino H, Ohizumi Y and Takemoto T. (1972a), Absorption, distribution, metabolism and excretion of insect-metamorphosing hormone ecdysterone in mice, I. *Yakugaku Zasshi*; 92:945-50.
- Hikino H, Ohizumi Y and Takemoto T. (1972b). Absorption, distribution, metabolism and excretion of insect-metamorphosing hormone ecdysterone in mice, II. *Chemical Pharmaceutical Bulletin*; 20:2454-8.
- Hirono I, Sasaoka I and Shimizu M. (1969). Effect of insect-molting hormones, ecdysterone and inokosterone, on tumor cells. *Gann*; 60:341-2.
- Kholodova YD. (2001) *Ukr. Biokhim. Zh.*; 73(3):21. (Article not seen; cited by Bathori & Pongracz 2005.)
- Khushbaktova ZA, Syrov VM and Batirov KH. (1991). *Khimiko-Farmatsevticheskii Zhurnal*; 25(4):53. (Article not seen; cited by Bathori & Pongracz 2005.)
- Kibrik ND and Reshetnyak JA. (1996). *Ear. Neuropsychopharm.*; 6(S4):167. (Article not seen; cited by Bathori & Pongracz 2005.)
- Klein R. (2004). Phytoecdysteroids. *Journal of the American Herbalists Guild*; Fall/Winter 2004:18-28.

- Konovalova NP, Mitrokhin YI, Volkova LM, Sidorenko LI and Todorov IN. (2002). Ecdysterone modulates antitumor activity of cytostatics and biosynthesis of macromolecules in tumor-bearing animals. *Izvestiia Akademii Nauk. Serii Biologicheskaja*; (6):650-8.
- Kuzmitskii BB, Golubeva MB, Konoplia NA, Kovganko NV, Akhrem AA. (1990). New possibilities in searching for immunomodulators among compounds with a steroid structure. *Farmakologiya i Toksikologiya*; 53(3):20-2. (Abstract only; article in Russian.)
- Lafont R and Dinan L. (2003). Practical uses for ecdysteroids in mammals including humans: and update. *Journal of Insect Science*; 3(7):1-30.
- Lafont R, Girault JP and Kerb U. (1988). Excretion and metabolism of injected ecdysone in the white mouse. *Biochemical Pharmacology*; 37:1174-7.
- Lagova ND and Valueva IM. (1981). Effect of ecdysterone isolated from *Rhaponticum carthamoides* on the growth of experimental tumors. *Eksperimentalnaya Onkologiya*; 3(4):69-71. (Abstract only; article in Russian.)
- Matsuda H, Kawaba T and Yamamoto Y. (1970). Pharmacological studies of insect metamorphosing steroids from *Achyranthis radix*. *Nippon Yakubutsugaku Zasshi (Folia Pharmacologica Japonica)*; 66:551-63.
- Mironova VN, Kholodova YD, Skachkova TF, Bondar OP, Datsenko ZM and Govseeva NN. (1982). Hypo Cholesterolemic Action Of Phyto Ecdysones In Experimental Hyper Cholesterolemia In Rats. *Voprosy Meditsinskoi Khimii*; 28(3):101-5. (Abstract only; article in Russian.)
- Ogawa S, Nishimoto N and Matsuda H. (1974) Pharmacology of ecdysones in vertebrates. In: *Invertebrate Endocrinology and Hormonal Heterophylly* (Ed. Burdette WJ). Springer-Verlag, Berlin; 341-344.
- Osipova SO, Islamova ZI, Syrov VN, Badalova NS and Khushbaktova ZA. (2002). Ecdysten in the treatment of giardiasis. *Meditsinskaja Parazitologiya i Parazitarnye Bolezni*; (1):29-33. (Abstract only; article in Russian.)
- Portugalov SN, Panyushkin VV and Abramova TF. (1996). *Nautsno. Tear.*; 7(9):1. (Article not seen; cited by Bathori & Pongracz 2005.)
- Mirzaev YR, Syrov VN, Krushev SA and Iskanderova SD. (2000). *Eskp. Klin. Farmacol*; 63(4):35. (Article not seen; cited by Bathori & Pongracz 2005.)
- Saatov Z, Agzamkhodzhaeva DA and Syrov VN. (1999). *Chem. Nat. Comp*; J5:186. (Article not seen; cited by Bathori & Pongracz 2005.)
- Simakin SY. (1998). *Sci. Sports Bull.*; 2:1. (Article not seen; cited by Bathori & Pongracz 2005.)
- Simon P and Koolman J. (1989). Ecdysteroids in vertebrates: pharmacological aspects. In: *Ecdysone - from chemistry to mode of action* (Koolman J, editor). Georg Thieme Verlag, Stuttgart: 254-9.
- Slama K and Lafont R. (1995). Insect hormones-ecdysteroids: Their presence and actions in vertebrates. *European Journal of Entomology*; 92(1):355-77.
- Slama K, Koudela K, Tenora J and Mathova A. (1996). Insect hormones in vertebrates: anabolic effects of 20-hydroxyecdysone in Japanese quail. *Experientia*; 52(7):702-6.
- Saydakhmedova MM and Kuznetsova TA. (1999). Use of ecdysten for correction of nutritive status of children with persistent diarrhoea syndrome. *Uzbekiston Tibbiet Zhurnali*; 1:37-9. (Abstract only; article in Russian.)
- Stopka P, Stancl J and Slama K. (1999). Effect of insect hormone, 20-hydroxyecdysone on growth and reproduction in mice. *Acta Societatis Zoologicae Bohemicae*; 63(3):367-78.

- Syrov VN. (1984). Mechanism Of Anabolic Action Of Phytoecdysteroids In Mammals. *Biologicheskie Nauki (Moscow)*; 11:16-20. (Abstract only; article in Russian.)
- Syrov VN. (2000). Comparative experimental investigation of the anabolic activity of phytoecdysteroids and steranabols. *Pharmaceutical Chemistry Journal*. 34(4):193-7.
- Syrov VN, Nabiev AN and Sultanov MB. (1986). Action of phytoecdysteroids on the bile-secretory function of the normal liver and in experimental hepatitis. *Farmakologiya i Toksikologiya*; 49(3):100-3. (Abstract only; article in Russian)
- Syrov VN, Tashmukhamedova MA, Khushbaktova ZA, Mirtalipov DT and Mamatkhanov AU. (1992). Effect of phytoecdysteroids and nerobol on parameters of carbohydrate and lipid metabolism and phospholipid spectrum of liver mitochondrial membrane in experimental diabetes mellitus of rats. *Ukrainskii Biokhimicheskii Zhurnal*; 64(4):61-7. (Abstract only; article in Russian.)
- Syrov VN, Khushbaktova ZA, Komarin AS, Abidov AB, Pechenitsyna TV and Aripkhodzhaeva FA. (2004). Experimental and clinical evaluation of the efficacy of ecdysten in the treatment of hepatitis. *Eksperimentalnaya i Klinicheskaya Farmakologiya*; 67(5):56-9. (Abstract only; article in Russian.)
- Tsitsimpikou C, Tsamis GD, Siskos PA, Spyridaki MH and Georgakopoulos CG. (2001). Study of excretion of ecdysterone in human urine. *Rapid Communications in Mass Spectrometry*; 15(19):1796-801.
- Uchiyama M. and Yoshida T. (1974). Effect of ecdysterone on carbohydrate and lipid metabolism. In: *Invertebrate Endocrinology and Hormonal Heterophylly* (Ed Burdette WJ), Springer-Verlag, Berlin; 401-16.
- Yoshida T, Otaka T, Uchiyama M and Ogawa S. (1971). Effect of ecdysterone on hyperglycemia in experimental animals. *Biochemical Pharmacology*; 20:3263-8.

**Certificate of analysis**

Rocky Mountain Chemical Company, Division of All American Pharmaceutical & Natural Foods Corporation

2019 Main Street Billings, MT 59105 U.S.A.

406-245-5793 Fax 406-252-1811

[www.allamericanpharmaceutica.com](http://www.allamericanpharmaceutica.com) XXXXXXXXXXXX

Certificate of Analysis

Customer: NSF  
 Item: 20-Hydroxy-ecdysterone  
 Lot #: 1541541  
 Code: RM-1541  
 Mfg. Date: January 2005  
 Exp. Date: January 2009  
 Date: 05-24-05

TEST	SPECIFICATIONS	RESULTS
1). Loss on Drying	1.0%	Conforms
2). Solution	Brown	Conforms
3). Residue on Ignition	>5%	Passes
4). Heavy Metals (as Pb)	<10 ppm	Passes
5). Assay	95.0% min	99.90%
6). Arsenic	<3 ppm	<3 ppm
7). Botanical Source	Vega Root Extract	
8). Active constituent	20-Hydroxy-ecdysterone	60.00%
Microbiological		
A). <i>Standard Plate Count</i>	3000/g max	Conforms
B). <i>Yeast/Mold</i>	50/g max	Conforms
C). <i>Coliform</i>	Negative/g	Negative
D). <i>E. Coli</i>	Negative/g	Negative
E). <i>Salmonella</i>	Negative/g	Negative
F). <i>Listeria</i>	Negative/g	Negative

XXXXXXXXXXXX

Quality Control Supervisor

### Supplier's data sheet

(Text reproduced here as printed in supplier's data sheet)

Chem-Base (Nantong) Laboratories Co., Ltd.

20-Hydroxyecdysone

Supplier: Chem-Base (Nantong) Laboratories Co., Ltd.

Total Ecdysterones is extracted from natural plant *Cyanotis vaga*.

Specification is as follows,

Appearance: Light yellow powder

Loss on drying: 5.0% max.

Residue of ignition: 1% max.

20-Hydroxyecdysone: 90% min. (UV)

( $\beta$ -Ecdysone)

Technological Process:

Raw Material -> Immersed in water -> Filtered -> Concentrated in Vacuum

Subsidence in Alcohol -> Filtered -> Concentrated in Vacuum -> Kill Bacteria

Dry -> Powder.

Note:

1. Formula  $C_{27}H_{44}O_7$
2. Product particle size is 80.

**TGA Library literature search strategy for Ecdysterone**

Search conducted on 25th August 2005

TGA Library was asked to conduct literature searches covering the medicinal use, safety and potential toxicity of the ingredient ecdysterone, both in traditional medicine and in current use. Search terms were identified from relevant literature already available, pharmacopoeias and materia medica.

The search was conducted in several parts:

1. An initial search of the ChemID database was conducted to identify synonyms and related substances. This record was sent to the client
2. A search of Medline (1966 to date) and Embase databases were searched together on the Ovid system.

The search strategy was:

- 1 exp ECDYSTERONE/ (1379)
- 2 5289-74-7.rm. (1380)
- 3 ecdysten.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, nm] (6)
- 4 ecdypure.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, nm] (0)
- 5 commisterone.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, nm] (2)
- 6 crustecdysone.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, nm] (7)
- 7 ecdysterone.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, nm] (6)
- 8 isoinokosterone.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, nm] (0)
- 9 polypodine A.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, nm] (15)
- 10 viticosterone.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, nm] (4)
- 11 beta-ecdysone.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, nm] (82)
- 12 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (1467)
- 13 exp LEUZEAE/ (5)
- 14 rhaponticum carthamoides.mp. (18)
- 15 12 or 13 or 14 (1482)
- 16 (toxicity\$ or safe\$ or adverse or trial or trials or interaction or pharmacokinetic\$ or dose or dosage or synthesis or extraction).mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, nm] (3773682)
- 17 15 and 16 (482)
- 18 remove duplicates from 17 (417)

3. A search of the RTECS (Registry of Toxic effects of Chemical Substances) was searched by the name of the substance Ecdysterone, and 19 records was located.
4. A search of BIOSIS and CABI on Dialog Datastar for all available years. The search terms were as follows:

ecdysterone or ecdysten or beta-ecdysone AND toxicity or toxic or safe or safety or adverse or trial or trials or interaction or pharmacokinetic or pharmacokinetics or dose or dosage

There were 374 results retrieved.

5. A multifile search of DIALOG databases (all years available for each file).  
Dialindex was searched in the medicine and alternative medicine database clusters to identify relevant further files for searching. The following were selected:

SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1951-2005/Aug W3

(c) format only 2005 Dialog

File 73:EMBASE 1974-2005/Aug 24

(c) 2005 Elsevier Science B.V.

File 5:Biosis Previews(R) 1969-2005/Aug W2

(c) 2005 BIOSIS

File 34:SciSearch(R) Cited Ref Sci 1990-2005/Aug W2

(c) 2005 Inst for Sci Info

File 35:Dissertation Abs Online 1861-2005/Jul

(c) 2005 ProQuest Info&Learning

File 144:Pascal 1973-2005/Aug W2

(c) 2005 INIST/CNRS

File 149:TGG Health&Wellness DB(SM) 1976-2005/Aug W2

(c) 2005 The Gale Group

File 156:ToxFile 1965-2005/Aug W3

(c) format only 2005 Dialog.

File 159:Cancerlit 1975-2002/Oct

(c) format only 2002 Dialog.

File 162:Global Health 1983-2005/Jul

(c) 2005 CAB International

File 399:CA SEARCH(R) 1967-2005/UD=14309

(c) 2005 American Chemical Society

File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec

(c) 1998 Inst for Sci Info

The search strategy was:

S (ECDYSTERONE OR ECDYSTEN OR BETA-ECDYSONE) AND (TOXICITY OR  
TOXIC OR SAFE OR SAFETY OR ADVERSE OR TRIAL OR TRAILS OR  
INTERACTION O

R PHARMACOKINETIC OR PHARMACOKINETICS OR DOSE OR DOSAGE)

Completed processing all files

4523 ECDYSTERONE

17 ECDYSTEN

196 BETA-ECDYSONE

2240533 TOXICITY

838231 TOXIC

482793 SAFE

982169 SAFETY

2502543 ADVERSE

1409862 TRIAL  
11512 TRAILS  
2930533 INTERACTION  
225750 PHARMACOKINETIC  
928232 PHARMACOKINETICS  
3507928 DOSE  
1719257 DOSAGE  
S1 608 (ECDYSTERONE OR ECDYSTEN OR BETA-ECDYSONE) AND  
(TOXICITY  
OR TOXIC OR SAFE OR SAFETY OR ADVERSE OR TRIAL OR TRAILS  
OR INTERACTION OR PHARMACOKINETIC OR PHARMACOKINETICS  
OR  
DOSE OR DOSAGE)  
RD  
S2 438 RD (unique items)  
S S2 FROM 155,73,5  
S3 343 S2 FROM 155,73,5  
S S2 NOT S3  
438 S2  
343 S3  
S4 95 S2 NOT S3

95 results were located.

A complete list of databases searched and titles retrieved was supplied to the evaluator for scrutiny.

XXXXXXXXXXXX

XXXXXXXXXXXX

Therapeutic Goods Administration