Parabens: Human health tier II assessment

24 April 2015

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Benzoic acid, 4-hydroxy-, propyl ester	94-13-3
Benzoic acid, 4-hydroxy-, butyl ester	94-26-8
Benzoic acid, 4-hydroxy-, methyl ester	99-76-3
Benzoic acid, 4-hydroxy-, ethyl ester	120-47-8
Benzoic acid, 4-hydroxy-, 1-methylethyl ester	4191-73-5
Benzoic acid, 4-hydroxy-, 2-methylpropyl ester	4247-02-3
Benzoic acid, 4-hydroxy-, methyl ester, sodium salt	5026-62-0
Benzoic acid, 4-hydroxy-, methyl ester, potassium salt	26112-07-2
Benzoic acid, 4-hydroxy-, ethyl ester, sodium salt	35285-68-8
Benzoic acid, 4-hydroxy-, propyl ester, sodium salt	35285-69-9

Chemical Name in the Inventory	CAS Number
Benzoic acid, 4-hydroxy-, ethyl ester, potassium salt	36457-19-9
Benzoic acid, 4-hydroxy-, butyl ester, sodium salt	36457-20-2
Benzoic acid, 4-hydroxy-, propyl ester, potassium salt	84930-16-5

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:**www.nicnas.gov.au**

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ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemicals in this assessment have been grouped as a result of their similar chemical structure and industrial uses. The parabens are esters of para-hydroxybenzoic acid and have been used extensively as antimicrobial preservatives in a number of applications for over 80 years (Cashman & Warshaw, 2005). Individual parabens are often used in combination with other parabens or other preservatives to provide protection against a broad range of microorganisms (Charnock & Finsrub, 2007). The parabens possess similar antimicrobial activity; however, they have differences in lipophilicity, giving rise to different physicochemical properties, such that a mixture of parabens often provides better protection than when individual parabens are used in isolation (Charnock & Finsrub, 2007).

The chemcials in this group will be referred to by their common names throughout this assessment. Their respective CAS numbers are listed below:

Methylparaben (CAS No. 99-76-3)

Ethylparaben (CAS No. 120-47-8)

Propylparaben (CAS No. 94-13-3)

Butylparaben (CAS No. 94-26-8)

Isopropylparaben (CAS No. 4191-73-5)

Isobutylparaben (CAS No. 4247-02-3)

Sodium methylparaben (CAS No. 5026-62-0)

Sodium ethylparaben (CAS No. 35285-68-8)

Sodium propylparaben (CAS No. 35285-69-9)

Sodium butylparaben (CAS No. 36457-20-2)

Potassium methylparaben (CAS No. 26112-07-2)

Potassium ethylparaben (CAS No. 36457-19-9)

Potassium propylparaben (CAS No. 84930-16-5)

Import, Manufacture and Use

Australian

The following Australian industrial uses were reported under previous mandatory and/or voluntary calls for information.

The chemicals have reported cosmetic use as antimicrobial preservatives.

Propylparaben (CAS No. 94-13-3) and methylparaben (CAS No. 99-76-3) have reported domestic or commerical use in surface coatings.

The chemicals have reported non-industrial uses in Australia as antimicrobial preservatives in food (FSANZ) and pharmaceuticals (TGA, 2007).

International

The following international uses have been identified through:

- the European Union (EU) Registration, Evaluation, Authorization and Restrictions of Chemicals (REACH) dossiers;
- Galleria Chemica;
- the Substances and Preparations in Nordic countries (SPIN) database;
- the European Commission Cosmetic Ingredients and Substances (CosIng) database;
- the United States (US) Personal Care Product Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary;
- the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); and
- various international assessments (NTP, 2005; SCCS, 2013).

The chemicals have reported cosmetic use including as antimicrobial preservatives.

The chemicals have reported domestic use, including as preservatives in:

- paints, lacquers and varnishes (sodium methylparaben, ethylparaben, methylparaben, propylparaben);
- adhesives and binding agents (methylparaben, propylparaben);
- cleaning agents (isopropylparaben, ethylparaben, methylparaben, propylparaben); and
- surface treatments (isopropylparaben, ethylparaben, methylparaben).

The chemicals have reported commercial uses, including in:

- fuel additives (ethylparaben);
- dehydrating agents (ethylparaben); and
- reprographic agents (ethylparaben, butylparaben, propylparaben).

The chemicals have reported site limited uses, including as stabilising agents (sodium propylparaben, sodium ethylparaben and sodium methylparaben).

The chemicals have reported non-industrial use, including as antimicrobial preservatives in pharmaceuticals and food.

Restrictions

Australian

The chemical methylparaben is listed in the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) in Appendix B—Substances considered not to require control by scheduling.

The other chemicals in this assessment are not listed in the SUSMP.

International

European Union: Use of 4-hydroxybenzoic acid, its salts and esters in cosmetics in the EU is subject to the restrictions described in EU Regulation Annex VI (Part 1), Reference #12. These chemicals may be used in cosmetics and personal care products at a maximum concentration of 0.4 % (acid) for a single ester and 0.8 % (acid) for mixtures of esters (CosIng).

Existing Worker Health and Safety Controls

Hazard Classification

The chemicals are not listed on the Hazardous Substances Information System (HSIS) (Safe Work Australia).

Exposure Standards

Australian

No specific exposure standards are available.

International

No specific exposure standards are available.

Health Hazard Information

Parabens are used extensively as preservatives in cosmetics and foods. Concern has been raised over the safety of their use as they have been found in biopsied breast carcinomas in humans (Darbre et al., 2004). There is also evidence to suggest parabens could play a role in endocrine dysfunction (Golden et al., 2005). Despite these reports, there is no empirical evidence that parabens can adversely affect human health.

Toxicokinetics

Absorption, metabolism, distribution and excretion

Oral exposure to the parabens results in rapid absorption via the gastrointestinal tract. The parabens undergo hydrolysis to p-hydroxybenzoic acid, which is then conjugated, and rapidly excreted in the urine (NTP, 2005).

Methylparaben was assessed in an in vitro metabolic study. Microsomal and cytosolic proteins from skin and liver samples, collected from humans and miniature pigs, were incubated with methylparaben at a concentration of 100 µM for one hour at 37 °C. Hydrolysis was assessed using high performance liquid chromatography. The chemical was found to be hydrolysed by both liver and skin microsomal/cytosolic fractions of humans and miniature pigs. The hydrolysis by human esterases was more rapid than that observed for miniature pigs (REACHa).

The metabolism and absorption of methylparaben was assessed in a study performed according to the Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 417 (toxicokinetics). Rats (six/sex/group) were topically administered a single dose of the radiolabelled test chemical at 100 mg/kg bodyweight (bw). Blood was taken at 0, 0.5, 1, 2, 4, 8, 12, 22 and 24 hours post-treatment. Urine and faeces were examined for up to 168 hours post-treatment. Under these test conditions, 14 and 26 % of the material was absorbed in males and females, respectively. The test chemical was completely metabolised to 4-hydroxybenzoic acid and its conjugates (REACHa).

The absorption, distribution, metabolism and excretion of methylparaben were assessed in canines. For absorption and excretion, a comparison was made between oral and intravenous administration of the chemical. Three fasted dogs were intravenously dosed with the chemical at 50 mg/kg bw. Blood and urine samples were collected and assessed for metabolites of the chemical. In parallel experiments, dogs were dosed orally with the chemical at 1000 mg/kg bw. Dogs were also infused intravenously with the test chemical at a rate of 2 mg/kg bw/min (until a total of 100 mg/kg bw was administered) to assess the distribution of the chemical. High plasma and urinary concentration of the methylparaben metabolite, conjugated p−hydroxybenzoic acid, and free methylparaben were observed, indicating that hydrolysis of the ester linkage and metabolic

conjugation represent the main pathways of methylparaben metabolism. The chemical was found to be preferentially distributed to the brain, spleen, liver, kidneys and plasma. Excretion of the test material was almost complete within 48 hours of administration (REACHa).

The toxicokinetics of ethylparaben have also been assessed in a conventional study in male Wistar rats. ^{14}C-labelled test material was injected intravenously (via the femoral vein) into two rats at 2 mg/kg bw. Urine and bile samples were collected periodically for five hours. The test chemical was almost completely metabolised and the following degradation products were present in the urine and bile of these animals: p-hydroxybenzoic acid, p-hydroxyhippuric acid, p-hydroxybenzoyl glucuronide and p-carboxyphenyl sulfate. Five hours after dosing, 91.3 % of the dose had been excreted, primarily in the urine (REACHb).

The metabolism and excretion of ethylparaben was assessed in an older study. Three dogs were administered the test material by intravenous injection at 50 mg/kg bw. After 48 hours, excretion of the test chemical and its metabolites was investigated. The major portion of the test material was recovered in urine as free p-hydroxybenzoic acid and as a conjugate with glucuronic acid, with 70 % of the administered dose excreted in urine within 48 hours (REACHb).

In dogs administered butylparaben via intravenous injection at 50 mg/kg and oral administration at 1000 mg/kg bw, 48 and 40 % of the dosed chemical was recovered in urine, respectively, within 30 hours. In a study in which dogs were dosed with the test material via intravenous injection at 100 mg/kg bw, the chemical was shown to be distributed to the brain, spleen, pancreas, liver and kidneys. Studies have also demonstrated that butylparaben can be absorbed dermally, hydrolysed in the skin or retained in the epidermis (NTP, 2005).

Dermal absorption

The dermal absorption of these chemicals is highly relevant given their use in cosmetic products.

A study was conducted according to OECD TG 428 (skin absorption: in vitro method) to assess the dermal absorption of methylparaben. Both human and rat skin samples were used in this study. Specimens were incubated with radiolabelled test material in solution (0.8 %) for 24 hours, with an approximate dose of 65 µg/cm^2. A greater amount of total radioactivity penetrated through the human skin (79.36 %) compared with rat skin (59.94 %). Notably, there was a greater portion of the test chemical metabolised in the rat skin than the human skin (53.9 and 35.1 %, respectively) (REACHa).

In another study conducted according to the OECD TG 428, skin specimens from humans and miniature pigs (three/species) were incubated with methylparaben at 25 µg/cm^2 for 24 hours. An average of 33.4 and 38.6 % of the chemical was absorbed through the human and miniature pig skin, respectively (REACHa).

The dermal absorption of ethylparaben was assessed in an OECD TG 428 study. Human skin specimens were exposed to the test chemical at 25 µg/cm^2 for 24 hours. Absorption of the chemical was reported to be 76.1 % under these conditions (REACHb).

Acute Toxicity

Oral

The chemicals have low acute toxicity based on results from animal tests following oral exposure. The median lethal dose (LD50) for all tested chemicals in the group is >2000 mg/kg bw. Observed sub-lethal effects included lethargy and prone posture.

Methylparaben was assessed for acute oral toxicity in accordance with OECD TG 401 (acute oral toxicity). Male Sprague Dawley (SD) rats (five/group) were administered the test chemical by gavage at 100, 500, 1000, 2000, 3000 or 4000 mg/kg bw. Mortality rates for these doses were 0/5, 0/5, 1/5, 2/5, 4/5 and 4/5, respectively. Effects, including reddened gastric mucosa and pulmonary congestion were noted in animals that died. Under the conditions of this study, an oral LD50 of 2100 mg/kg bw was determined (REACHa).

In a study conducted according to the OECD TG 401, 10 SD rats were administered methylparaben as a single dose of 5000 mg/kg bw. No mortalities were recorded. Therefore, on the basis of this result, an oral LD50 of >5000 mg/kg bw was determined

(REACHa).

Methylparaben was assessed in another OECD TG 401 study in male ICR mice. Animals were administered the test chemical at 2600, 3200, 4000, 4800 or 5600 mg/kg bw by gavage and observed for seven days post-treatment. No mortalities were recorded at any of the doses assessed. Therefore, under these test conditions, an oral LD50 of >5600 mg/kg bw in ICR mice was determined (REACHa).

Male dogs were administered methylparaben in an oral acute toxicity study. Despite some limitations in the experimental design, an oral LD50 of >2000 mg/kg bw was reported. Methylparaben has also been shown to have low acute toxicity in guinea pigs (3000 mg/kg bw) and rabbits (>2000 mg/kg bw) (REACHa).

Ethylparaben was assessed in a study conducted according to the OECD TG 401. Wistar rats of both sexes were administered the test chemical at 1000, 3100 or 5000 mg/kg bw by oral gavage and observed for a period of 14 days. Based on the death of 4/10 animals in both the 3100 and 5000 mg/kg bw groups, an LD50 of >3100 mg/kg bw was determined. Sub-lethal effects included lethargy and prone posture (REACHb).

Propylparaben was assessed in an OECD TG 401-compliant study. Male and female Wistar rats were administered the test chemical by oral gavage at 5000 mg/kg bw. No mortalities occurred during the 14-day observation period and an oral LD50 of >5000 mg/kg bw was determined. (REACHc).

Propylparaben was shown to possess very low acute toxicity. Five female albino rats (strain not specified) were administered the test material at 15000 mg/kg bw by oral gavage, in an OECD TG 401 study. No signs of toxicity were observed in any animals and no mortalities occurred during the seven-day observation period. An oral LD50 of >15000 mg/kg bw was determined (REACHc).

Sodium methylparaben was assessed in an OECD TG 401 study. Wistar rats of both sexes (10/group) were dosed orally with the test chemical at 3100 or 5000 mg/kg bw. During a 14-day observation period, no mortality was recorded in the lower dose group, while two males died in the higher dose group. Sub-lethal effects included prone posture and sedation. An oral LD50 of >5000 mg/kg bw was determined (REACHd).

The US National Toxicology Program (NTP) has reviewed oral toxicity data for butylparaben and sodium butylparaben. They reported oral LD50 values of 5000 mg/kg and 950 mg/kg bw in mice (strain and sex not specified) for butylparaben and sodium butylparaben, respectively (NTP, 2005).

No data are available for the other chemicals in this group.

Dermal

The chemicals have low acute toxicity based on results from animal tests following dermal exposure for several chemicals in the group. The LD50s were >2000 mg/kg bw.

A cosmetic product containing methylparaben at a concentration of 0.2 % was assessed for dermal acute toxicity in albino rabbits of both sexes. Doses of 2.0 mL/kg were applied to intact and abraded skin and occluded for 24 hours. No evidence of toxicity was reported during a 14-day observation period (Anderson, 2008).

Butylparaben was assessed in a dermal acute toxicity study, reviewed by the NTP (NTP, 2005). No experimental details were provided; however, a dermal LD50 of >2000 mg/kg was determined in a study using rabbits of both sexes (strain and number not specified) (NTP, 2005).

No data are available on the other chemicals in this group.

Inhalation

Methylparaben was shown to be mildly ciliotoxic in male Wistar rats at an inhaled concentration of 1.18 mM during a four-hour exposure (Anderson, 2008). Few experimental details were provided, including the actual inhaled dose.

No data are available for the other chemicals in this assessment.

Observation in humans

Few data are available on the acute toxicity of parabens in humans. However, it is generally thought that the parabens are not acutely toxic. It has been reported that inhalation exposure to butylparaben results in irritation of the respiratory tract, causing coughing and dyspnoea (NTP, 2005).

Corrosion / Irritation

Skin Irritation

The chemical, sodium methylparaben, was reported to slightly irritate skin in an in vitro study. The other chemicals in this group which have been tested were not irritating to skin.

Methylparaben (0.1 mL) was applied to the shaved skin of nine albino rabbits, covered with an occlusive patch and left for 24 hours. During the 72-hour observation period, no evidence of skin irritation was noted and it was determined that the chemical was not a skin irritant (REACHa).

Ethylparaben was tested in a study similar to the OECD TG 404 (acute dermal irritation/corrosion). The moistened chemical (0.5 g) applied to the clipped skin of three New Zealand White rabbits under a semi-occlusive patch for four hours. The animals were observed at regular intervals for seven days. No erythema or oedema developed at the test sites at any observation point. Therefore, under these test conditions, ethylparaben did not act as a skin irritant (REACHb).

In another skin irritation study, 0.1 mL of a 100 % solution of ethylparaben was applied to a 5 cm^2 area on the back of nine white albino female rabbits for 24 hours under an occlusive patch. The animals were observed for 72 hours. No evidence of erythema or oedema was observed and, as a result, the test material was determined not to be an irritant (REACHb).

Sodium methylparaben was assessed for dermal irritation in a study conducted according to the OECD TG 431 (in vitro skin corrosion: human skin model test). This test used an organotypic reconstituted three-dimensional model of human epidermis. The tissue was incubated with the test chemical in solution at 1000 mg/mL, for three or 60 minutes. No evidence of corrosion was observed in this culture system. Therefore, under these test conditions, sodium methylparaben was determined not to be corrosive (REACHd).

Sodium methylparaben was assessed in another in vitro dermal irritation study conducted according to the OECD TG 439 (in vitro skin irritation: reconstructed human epidermis test method). The chemical was applied to the reconstructed human epidermis at a concentration of 1000 mg/mL, for 60 minutes. The chemical reduced cell viability and, therefore, the chemical was determined to be irritating (REACHd).

No data are available for the other chemicals in this group.

Eye Irritation

Sodium methylparaben is reported to slightly irritate the eyes in animal studies. The other chemicals in this group that have been tested were not irritating to the eyes.

Methylparaben was assessed for ocular irritation in a non-guideline in vivo study where 0.1 mL of an undiluted solution of the test chemical was instilled into one eye of each of six New Zealand White rabbits. Animals were assessed at regular intervals for seven days. The chemical produced very slight irritation (irritation score of 1/110), which was fully reversible (REACHa).

Ethylparaben was assessed in a study conducted in accordance with the OECD TG 405 (acute eye irritation/corrosion) where 0.1 mL of an undiluted test chemical solution was instilled into one eye of each of three female New Zealand White rabbits. No evidence of ocular irritation was observed and, as a result, the test chemical was determined to be non-irritating (REACHb).

Propylparaben was assessed for eye irritation in a study according to the OECD TG 405 where 0.1 g of the test chemical was introduced into one eye of each of three New Zealand White rabbits. The chemical caused mild, transient ocular changes

including reddening of the sclerae and conjunctivae as well as discharge. Ocular damage fully resolved within seven days of chemical application. No abnormal findings were observed in the cornea of any animals at any of the examinations; the chemical was found to be slightly irritating to the rabbit eye (REACHc).

In an in vitro study using propylparaben, three freshly harvested bovine corneas were exposed to 0.75 mL of a 20 % suspension of the chemical in saline, for 240 minutes. Following removal of the test chemical, corneal opacity and permeability were assessed. Compared with the negative control, the test chemical caused a slight increase in corneal opacity, but no increase in permeability. The chemical was considered not to be corrosive or a severe irritant (REACHc).

Sodium methylparaben was assessed in an in vitro ocular irritation study. Six New Zealand White rabbits received 0.1 mL of the test chemical in solution (concentration not reported) in one eye. After the eight-day observation period, corneal and conjunctival swelling were not fully reversible in two animals, and iridial effects and conjunctival erythema were not reversible in three animals. On the basis of these findings, sodium methylparaben is considered to be an ocular irritant (REACHd).

No data are available for the other chemicals in the group.

Observation in humans

Butylparaben is reported to cause irritation, redness and pain when in contact with the eyes. It has also been suggested that the chemical causes skin irritation. Respiratory irritation could also occur following inhalation exposure to butylparaben. Symptoms included coughing and shortness of breath (NTP, 2005).

Photocontact sensitisation and phototoxicity tests on product formulations containing methyl, propyl and butylparaben (at concentrations up to 0.8 %) gave no evidence of photoreactivity. However, exposure to butylparaben and sunlight has caused excessive hyperpigmentation. Butylparaben also reportedly exacerbated dermatitis in people with the condition (NTP, 2005).

Parabens are not thought to be irritating in people with normal, undamaged skin (Soni et al., 2005).

Sensitisation

Skin Sensitisation

The chemicals were not found to cause dermal sensitisation when tested according to the OECD TG 406. However, butylparaben could possess some sensitising potential, although the human data do not support this.

Methylparaben has been assessed for its potential to cause skin sensitisation. In an in vivo study, 20 Pirbright White guinea pigs (10/sex) received the test chemical via intradermal injection at 0.1 %, followed by an epidermal challenge at a concentration of 5 % (according to the Maurer optimisation test (Maurer et al., 1975)). The test chemical caused a positive response in some animals (4/20), but this result did not meet the criteria for a positive conclusion. Therefore, methylparaben is not considered to be a skin sensitiser (REACHa).

Methylparaben was assessed for skin sensitisation in a study (maximisation test) where male guinea pigs received 10 repeated intradermal injections for induction. Two weeks after the final injection, the animals received a single intradermal challenge injection at a concentration of 0.1 %. No positive reactions were observed in any of the animals; therefore, under these test conditions, the chemical was not considered to be a skin sensitiser (REACHa).

Ethylparaben was assessed in a skin sensitisation study conducted in accordance with the OECD TG 406 (skin sensitisation). Female Hartley guinea pigs were administered the test chemical via intradermal injection and epicutaneous application (induction phase). This was followed eight days later by epicutaneous application of the test chemical under an occlusive patch (challenge). Six animals died during the test period (including one negative control). However, all deaths were attributed to excessively tight occlusive-patch wrapping. No sensitising effect was observed in any of the animals. On the basis of these results, the test chemical was determined not to be a skin sensitiser (REACHb).

Butylparaben has been assessed for skin sensitisation. The chemical (at 0.1 %) was injected intradermally (three/week for three weeks) into the back and upper flanks of guinea pigs. A challenge dose was given to the animals two weeks later. Neither the

initial injections, nor the challenge injection generated any positive responses in the guinea pigs. On the basis of this result, butylparaben was deemed not to be a skin sensitiser. Similar results were obtained with sodium butylparaben (Matthews et al., 1956).

In another experiment, guinea pigs were injected intradermally with butylparaben (5 %) three times per week, for three weeks, along with Freund's complete adjuvant on days 1 and 10 of the study. Animals received a challenge dose, applied topically under occlusive patches (5 %), two weeks after the final induction injection. Six out of 20 animals exhibited reactions to the test chemical, the worst of which was spongiosis, squamous crust, and lymphocytic infiltration (NTP, 2005; Andersen, 2008).

Quantitative Structure Activity Relationship (QSAR) models can be used to predict the activities of data-poor chemicals, based on known relationships between chemical structures and biological activity that have previously been reported. QSAR analysis of sodium methylparaben gave no alerts for skin sensitisation (REACHd).

There are no available data for the other chemicals in this assessment.

Observation in humans

In a skin irritation and sensitisation study, 50 human volunteers were administered butylparaben at 5, 7, 10, 12 and 15 % to the skin on the back once daily for five consecutive days. No irritation was observed at a concentration of 5 % (Anderson, 2008). In a repeated insult patch test, the chemical was applied to the skin of 50 volunteers for 4–8 hours every other day for a total of 10 administrations. No evidence of irritation was observed. Reapplication of the test chemical three weeks later produced no signs of allergic irritation and thus the chemical was not considered a sensitiser (NTP, 2005).

Repeated Dose Toxicity

Oral

Considering the no observed effect levels (NOELs) available from long-term rat studies (ranging from 100–1000 mg/kg bw/day), and based on the treatment-related effects reported in various repeated dose toxicity studies, repeated oral exposure to the chemicals is not considered to cause serious damage to health.

Methylparaben was assessed for repeated dose toxicity according to the OECD TG 407 (repeated dose 28-day oral toxicity in rodents). Wistar rats (5 animals/sex/dose/group) were administered the test chemical by oral gavage at 50, 250 or 1000 mg/kg bw/day. No mortalities occurred during the study; although, two animals were euthanised in the high dose group due to their moribund appearance. Pathological findings in these animals included slight erosion of the gastric mucosa, slight red pulp atrophy of the spleen and moderate lymphoid atrophy of the thymus. Animals in the high dose group exhibited piloerection and/or hunched posture. These rats, along with one female from the 250 mg/kg bw/day group, had laboured respiration, rales and gasping. These signs were considered to be treatment-related. There were no significant changes noted in any other parameters assessed, including haematology, clinical chemistry, urinalysis, body weight, food consumption and ophthalmoscopy. Under these experimental conditions, a no observed adverse effect level (NOAEL) of 250 mg/kg bw/day was determined (REACHa).

A pre-guideline, 120-day repeated dose oral toxicity study was performed using methylparaben. Guinea pigs (six/group, sex not specified) were administered the test chemical (method not specified) at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 100 mg/day, for 120 days. No effects on food consumption, body weight or haematology were observed in any of the animals at any dose. On the basis of this finding, a NOEL of 100 mg/animal/day was determined (REACHa).

Rats of unspecified strain (six/group) were fed diets containing methylparaben at 2 % (equivalent to 900–1200 mg/kg bw/d) or 8 % (equivalent to 5500–5900 mg/kg bw/d) for 96 weeks. Food consumption and body weight gain were assessed, along with gross and histopathological changes. Animals in the low dose group did not show any adverse effects during the study period. Animals in the high dose group showed slower body weight gain than controls. No other substance-related effects were observed in this study (REACHa). No NOAEL was provided; however, on the basis of these findings a NOEL of 900–1200 mg/kg bw/day can be deduced.

In a study that assessed the repeated dose toxicity of methylparaben in mongrel dogs, animals were orally administered the test chemical at 500 mg/kg bw/day (two animals) or 1000 mg/kg bw/day (three animals), six days a week for up to 422 days. No toxicity-related signs were observed in any of the animals. There was no accumulation of the test chemical and blood and urine parameters were unchanged. At the conclusion of the study, the animals were reported to be in excellent condition. One female in the lower dose group was successfully mated and delivered a litter of healthy pups. As a result, a NOEL of 1000 mg/kg bw/day was determined (REACHa).

The effects of repeated oral exposure to ethylparaben have been studied in Wistar rats. Animals of both sexes (12/dose) were fed diets containing the test chemical at 2 % (equivalent to 900–1200 mg/kg bw/day) or 8 % (equivalent to 5500–5900 mg/kg bw/day) for 12 weeks. Food consumption, weight gain and pathological changes were assessed. Animals in the high dose group exhibited a slower rate of weight gain compared with controls. Depressed motor activity was also observed in this group. No pathological changes were observed in the kidney, heart, lung, spleen or pancreatic tissue. Mortalities associated with pulmonary pathology occurred in test and control animals and were not thought to be treatment-related (few experimental details were provided). On the basis of these findings, a NOAEL of 900–1200 mg/kg bw/day was determined (Matthews et al., 1956).

Male and female SD-JCL rats were administered ethylparaben in the diet at 0.2, 1.0 and 2.0 % (equivalent to approximately 120, 600 and 1200 mg/kg bw/day, respectively). No mortalities occurred during this study. There were no significant changes in food consumption, body weight or haematological parameters when compared with controls. There was a significant change in alkaline phosphatase serum concentrations in male animals; however, this was not dose-dependent. A NOAEL of ≥1200 mg/kg bw/day was determined (REACHb).

A study assessed propylparaben for repeated dose toxicity as well as reproductive and developmental toxicity in accordance with the OECD TG 422 (combined repeated dose toxicity study with the reproduction/developmental toxicity screening test). The test chemical was administered to male Wistar rats for 28 days and to females for 14 days before pairing, through the pairing and gestation periods until the F1 generation reached day four post-partum. Males were also dosed during the post-pairing phase of the experiment. Animals were fed diets containing the test chemical at 0, 1500, 4500 or 15000 ppm (equivalent to 98.0, 305.1 and 980.9 mg/kg bw/day (pre-pairing) and 59.3, 178.3 and 605.0 mg/kg bw/day (after pairing), respectively, for males and equivalent to 116.0, 341.9 and 1076.4 mg/kg bw/day (pre-pairing), 121.6, 349.2 and 1124.6 mg/kg bw/day (gestation) and 137.3, 431.8 and 1380.0 mg/kg bw/d (lactation), respectively, for females). No signs of toxicity were observed in males or females at any dose group. Under these test conditions, a NOAEL of 15000 ppm, was determined (REACHc).

Propylparaben has been assessed in a 96-week repeated dose toxicity study. Male and female Wistar rats were fed diets containing the chemical at 2 % (equivalent to 900–1200 mg/kg bw/day) and 8 % (equivalent to 5500–5900 mg/kg bw/day). Animals in the higher dose group exhibited reduced growth rate compared with controls. This trend was not evident in the lower dose group. No other treatment-related effects were reported. Histopathological examination of surviving animals revealed no significant abnormalities. Therefore, under these test conditions, a NOAEL of 900–1200 mg/kg bw/day (2 % in feed) was determined for propylparaben.

Butylparaben was fed to ICR/Jcl mice in food pellets at 0.6, 1.25, 2.5, 5 or 10 % (equivalent to 900, 1900, 3800, 7500, or 15000 mg/kg bw/day, respectively) for six weeks. Mortalities occurred in the highest dose group and significant atrophy of the lymphoid tissue in the spleen, thymus and lymph nodes occurred at doses higher than the lowest dose of 0.6 %. Multifocal degeneration of the liver was also observed (NTP, 2005).

Wistar rats were fed a diet containing butylparaben at 2 % or 8 % (equivalent to 2000 or 8000 mg/kg bw/day, respectively) for up to 12 weeks. All males in the high dose group died during the treatment period. Premature deaths also occurred in females in the high dose group. No toxic effects were observed in the low dose group (Matthews, 1956).

Another study examined the effects of repeated dosing of butylparaben and isobutylparaben in ICR/Jcl mice. Animals (50/sex/group) were administered the test chemical at 0.15, 0.3 or 0.6 % in the diet for 102 weeks. A high incidence of amyloidosis in the spleen, liver, kidneys and/or adrenal glands of the 45 % and 27 % of surviving males and females from the highest dose butylparaben group respectively, and 58 % and 33 % of males and females in the highest dose isobutylparaben group, respectively, were observed. An estimated daily maximum ingested dose of 40 mg/mouse for both chemicals was determined on the basis of this study (Inai et al., 1985).

No data are available for the other chemicals in this assessment.

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Based on the weight of evidence from the available in vitro and in vivo genotoxicity studies, the chemicals are not considered to be genotoxic. Several in vitro (bacterial reverse mutation and mammalian cell mutation) and in vivo (mammalian cell chromosome aberration, comet and dominant lethal) assays for gene mutation and clastogenicity were negative. Some in vitro genotoxicity tests indicated weakly positive results, but all in vivo tests were negative.

In vitro

A study using methylparaben was conducted in compliance with the OECD TG 471 (bacterial reverse mutation assay) using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 or TA1538, both in the absence and presence of a rat-liver-derived metabolic activation system (S9). The chemical did not cause an increase in the number of revertant colonies in any of the bacterial strains tested, either with or without metabolic activation at concentrations up to 50 µg/plate (REACHa).

Methylparaben was assessed in another bacterial reverse mutation assay conducted according to the OECD TG 471. The test chemical was incubated with *S. typhimurium* TA98, TA1535, TA1537 and TA1598 strains at concentrations of up to 10 mg/plate. The chemical did not induce any significant increases in revertant colonies in any of the strains tested at any concentration in the presence or absence of metabolic activation. Therefore, this chemical was considered not to be genotoxic under these test conditions (REACHa).

A chromosomal aberration study was performed using methylparaben. Chinese hamster lung fibroblasts (V79) were incubated with the test chemical at 0.125 mg/mL both in the presence and absence of S9 metabolic activation. The chemical was found to cause some chromosomal aberrations in the presence of metabolic activation. Therefore, methylparaben was considered to be slightly mutagenic with metabolic activation (REACHa).

A study assessing ethylparaben was conducted in compliance with the OECD TG 471, using *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537. Cells were incubated with the test chemical at doses ranging up to 5 mg/plate, both in the presence and absence of a metabolic activation system. The chemical did not induce any statistically significant increase in the frequency of revertant colonies in any of the strains tested, at any concentration tested, with or without metabolic activation. The test chemical was considered to be non-genotoxic (REACHb).

In a bacterial reverse mutation assay, which has not been described in great detail, propylparaben did not show any evidence of genotoxicity in *S. typhimurium* strains TA98 and TA100 or in the *Escherichia coli* WP2 strain, in the absence or presence of a metabolic activation system. Under these test conditions, the chemical was determined not to be genotoxic in vitro (REACHc).

Propylparaben was assessed in an in vitro genotoxicity study performed in compliance with the OCED TG 476 (in vitro mammalian cell gene mutation test). Chinese hamster lung fibroblasts (V79) were treated with the test chemical at concentrations ranging up to 448.0 µg/mL, both in the presence or absence of a metabolic activation system. No relevant and/or reproducible increase in the numbers of chromosomal aberrations was observed at any concentration tested, with or without metabolic activation (REACHc).

Isopropylparaben was negative in a bacterial reverse mutation assay using *S. typhimurium* strains TA92, TA94, TA98, TA1535, TA100, TA1537 at concentrations up to 1 mg/plate, in the presence or absence of metabolic activation (Anderson, 2008).

Isobutylparaben was also negative in a bacterial reverse mutation assay using *S. typhimurium* strains TA92, TA94, TA98, TA1535, TA100, TA1537 at concentrations up to 1 mg/plate, in the presence or absence of metabolic activation. Isobutylparaben was also negative for mutagenesis in a chromosomal aberration assay using a Chinese hamster fibroblast cell line (Anderson, 2008).

Butylparaben did not exhibit genotoxicity when assessed in a bacterial reverse mutation assay using *S. typhimurium* strains TA92, TA94, TA97, TA98, TA100, TA102, TA1535, TA1537, and TA2637 at concentrations up to 1000 mg/plate. The chemical was found to induce a minimal increase in polypoid cell production when incubated with Chinese hamster cells at 0.06 mg/L. However, the chemical was not mutagenic in fibroblast and ovary cells.

No data are available for the other chemicals in this assessment.

In vivo

Methylparaben has been assessed in an in vivo chromosome aberration assay conducted in compliance with the OECD TG 475 (mammalian bone marrow chromosome aberration test). Male SD rats were used across two experiments. Animals were given a single dose of the test material by gavage at 5, 50, 500 or 5000 mg/kg bw, or single doses on five consecutive days at the same levels. The test chemical did not cause any significant aberrations of the bone marrow cell chromosomes when compared with negative controls, indicating that the test chemical is not mutagenic (REACHa).

Methylparaben has been assessed for genotoxicity in vivo in a dominant lethal assay conducted in compliance with the OECD TG 478 (genetic toxicology: rodent dominant lethal test). Male SD rats were used across two experiments. Animals were given a single dose of the test material by gavage at 5, 50, 500 or 5000 mg/kg bw or single doses on five consecutive days at the same levels. The males were sequentially mated to two females per week for eight weeks. Females were euthanised 14 days following mating and uterine examinations were performed. Corpora lutea, early deaths, late foetal deaths and total implantations were assessed. No dose-response or time-trend patterns were observed in any of the parameters assessed, indicating that under these test conditions, methylparaben was not mutagenic (REACHa).

Butylparaben was assessed in an in vivo comet assay using ddY mice. The test chemical was administered to animals via gavage at 2000 mg/kg bw. There was no statistically significant increase in DNA damage observed in the stomach, colon, liver, kidneys, bladder, lungs, brain or bone marrow at three and 24 hours post-treatment. On the basis of this finding, butylparaben is not considered to be genotoxic (Sasaki et al., 2002).

No data are available for the other chemicals in this assessment.

Carcinogenicity

Based on the weight of evidence from the available well-conducted in vivo carcinogenicity studies, the chemicals in this assessment are not considered to be carcinogenic.

Methylparaben was assessed for carcinogenicity in a study conducted using male and female C57/BL/6, A/Jax and CF1 mice. This study encompassed three distinct experiments, which assessed the effect of single and multiple subcutaneous and intravenous injections at 2.5 mg/animal at weekly and monthly intervals. No carcinogenic effects were observed in any of the animals when compared with controls. On the basis of this finding, a NOEL for carcinogenicity of 2.5 mg/animal was determined (REACHa).

A wide ranging study was conducted to determine the carcinogenic effects of methylparaben in male and female mice and rats of various strains (mice: Swiss, Balb/c, a/He Jax, CF-1; rats: Fischer (F) 344). Across separate experiments, the potential carcinogenic effects from the route and frequency of administration, as well as age at administration, were assessed. The chemical was tested at doses up to 2.5 mg/kg bw/day in mice and 3.5 mg/kg bw/day in rats. No carcinogenic effects were observed in any of the animal strains tested. The investigators reported that the chemical had a NOEL for carcinogenicity of 2.5 mg/kg bw/day in male and female mice and a NOAEL for carcinogenicity of 3.5 mg/kg bw/day for male and female rats (REACHa).

In another study, the carcinogenic properties of methylparaben were evaluated in male and female F344 rats. Animals were administered the chemical twice weekly, via subcutaneous injection at 0, 0.6, 1.1, 2.0 or 3.5 mg/kg bw/day for six months. No statistically significant carcinogenic effects were observed at any of the doses tested. As a result of this finding, a NOEL for carcinogenicity of 3.5 mg/kg bw/day was determined (REACHa).

In mice fed butylparaben at 0, 0.15, 0.3 or 0.6 % in the diet for 106 weeks, tumour incidence was increased and time to tumour development was decreased in animals treated with the test chemical. However, the findings were not statistically significant (Inai et al., 1985).

No data are available on the other chemicals in this assessment.

Observations in humans

Concerns have been raised about the potential for parabens to induce or promote cancer growth. These concerns relate to the observation that parabens were present in 20 excised breast cancer tissue samples (Darbre et al., 2004) and the fact that parabens do exhibit weak oestrogenic activity (Golden et al., 2005) (see **Reproductive and developmental toxicity**). Despite these observations, no study has demonstrated a causal relationship between paraben exposure and carcinogenesis.

Reproductive and Developmental Toxicity

The chemical does not show specific reproductive or developmental toxicity. Any reproductive and developmental effects were generally only observed secondary to maternal toxicity.

Developmental toxicity

A study conducted in compliance with the OECD TG 414 (prenatal developmental toxicity) assessed methylparaben for developmental toxicity. Female Dutch-belted rabbits were artificially inseminated with semen from a proven donor buck and then administered the chemical via oral gavage on gestation day (GD) 6–18 at doses of 3, 14, 65, 300 mg/kg bw. Females were subjected to caesarean section on gestation day 29 and the numbers of corpora lutea, implantation sites, resorption sites and dead foetuses were recorded. No statistically significant patterns were seen and a NOEL of 300 mg/kg bw/day was determined for both maternal and developmental toxicity (REACHa).

Methylparaben was assessed in a developmental toxicity study conducted according to the OECD TG 414. Following mating with young adult males, female CD-1 mice were administered the test chemical via oral gavage on GD 6–15 at doses of 5.5, 25.5, 118.0 or 555.0 mg/kg bw/day. No statistically significant effects were observed in a number of parameters of maternal and developmental toxicity at any dose. A NOEL of 555.0 mg/kg bw/day was determined for both maternal and developmental toxicity (REACHa).

Two more studies, conducted according to the OECD TG 414, have been conducted to ascertain the developmental toxicity of methylparaben. In one study using Wistar rats, a NOEL of 550 mg/kg bw/day for both maternal and developmental toxicity was recorded. In a study in Golden hamsters, a NOEL of 300 mg/kg bw/day was determined for both maternal and developmental toxicity. In both of these studies, the NOELs were the highest doses tested (REACHa).

Ethylparaben was assessed in a developmental toxicity study. Following mating, pregnant Wistar rats were administered the test chemical on GD 8–15 in their feed at 0.1, 1 or 10 % (approximately equivalent to 54–63, 517–658 and 2970–3260 mg/kg bw/day). Numerous parameters of maternal and developmental toxicity were assessed. On the basis of decreased maternal food intake, a NOAEL of 517–658 mg/kg bw/day was determined for maternal toxicity. On the basis of bone malformations, hydronephrosis and enlargement of the 3rd ventricle of the brain, a NOAEL of 517–658 mg/kg bw/day was determined for developmental toxicity (REACHb).

The developmental toxicity of propylparaben was investigated in a non-guideline study. Pregnant CF-1 mice were dosed with the chemical via subcutaneous injection, on GD 1–4, at 948.5 or 1084.0 mg/kg bw/day. Two days following the final dose, dams were euthanised, their uteri removed and the number of visible intrauterine implantation sites counted. No statistically significant toxic effects were observed and as a result, a NOAEL of 1084 mg/kg bw/day was determined for developmental toxicity (REACHc).

The developmental toxicity of butylparaben was assessed in several studies. In one study, the chemical was administered to pregnant SD rats from GD 6 through to postnatal day 20 at 100 or 200 mg/kg bw/day, via subcutaneous injection. A significant decrease in the proportion of pups born alive was observed in both groups. On the basis of this finding, a lowest observed effect level (LOEL) of 100 mg/kg bw/day was determined (Kang et al., 2002).

Neonatal Wistar rats were administered butylparaben by subcutaneous injection at 2 mg/kg bw/day on days 2–18 of life. No effect was observed in any developmental parameters including testis weight and morphology. On the basis of this finding, a NOEL of 2 mg/kg bw/day for developmental toxicity was determined (Fisher et al., 1999).

In another developmental toxicity study, butylparaben was administered by gavage to pregnant SD rats at 10, 100 or 1000 mg/kg bw on GD 6–19. In the high dose group, decreases in maternal weight gain were observed and maternal food

consumption was also significantly decreased over the dosing period. A NOAEL of 100 mg/kg bw/day was determined for maternal toxicity. No differences were seen in any of the developmental parameters assessed (including embryo viability, foetal weight, malformations) and a NOAEL of 1000 mg/kg bw/day was determined for developmental toxicity (NTP, 2005).

Reproductive toxicity

Male Crj:Wistar rats were administered ethylparaben at 0.1 or 1.0 % of their feed (equivalent to 103 and 1043 mg/kg bw/day) for eight weeks. No statistically significant differences or biologically relevant changes in body weights or testes, epididymis, prostate, seminal vesicle or preputial gland weights were observed. The NOAEL for fertility for ethylparaben under these test conditions was determined to be 1043 mg/kg bw/day (REACHb).

Propylparaben was assessed in a study assessing repeated dose toxicity as well as reproductive and developmental toxicity conducted in accordance with OECD TG 422 (See **Repeated dose toxicity** section). The chemical was administered to male Wistar rats for 28 days and to females for 14 days before pairing, through the pairing and gestation periods until the F1 generation reached day four post-partum. Males were also dosed during the post-pairing phase of the experiment. Animals were fed diets containing the test chemical at 0, 1500, 4500 or 15000 ppm (equivalent to 98.0, 305.1 and 980.9 mg/kg bw/d (pre-pairing) and 59.3, 178.3 and 605.0 mg/kg bw/d (after pairing), respectively, for males and equivalent to 116.0, 341.9 and 1076.4 mg/kg bw/d (pre-pairing), 121.6, 349.2 and 1124.6 mg/kg bw/d (gestation) and 137.3, 431.8 and 1380.0 mg/kg bw/d (lactation), respectively, for females). There were no statistically significant changes in any of the reproductive parameters assessed (sperm motility/morphology/viability, fertility, gestation period, corpora lutea count, implantation rate, post implantation loss, postnatal loss and litter size). There were no treatment-related findings in pups following birth, the first four days post-partum or at necropsy. Based on the findings from this study, a NOEL of 15000 ppm was determined for reproductive/developmental toxicity (REACHc).

Butylparaben was assessed for reproductive toxicity in a non-guideline study. In Crj:CD-1 mice administered the test chemical in their diet (at concentrations ranging from 0.01–1 %) for 10 weeks, epididymal weights were significantly increased when compared with controls. Statistically significant reductions in serum testosterone concentration were also observed. Although few experimental details were provided, the investigators reported a NOEL of 100 mg/kg bw/day (NTP, 2005).

No data are available for the other chemicals in this assessment.

Other Health Effects

Endocrine Disruption

Endocrine activity

The parabens possess oestrogenic activity, although at much lower potency than endogenously produced oestrogens. There are currently no established adverse outcome pathways for weak oestrogenic activity.

The oestrogenic activity of methylparaben towards the oestrogen receptors (ER) a and β was assessed using three reporter cell lines: HELN, HELN ERa and HELN ERβ. These cell lines were generated using HeLa cells, which do not express oestrogen receptors. Cells were incubated with the chemcial for 16 hours; with doses ranging up to 10 µM. Methylparaben was found not to exhibit any oestrogenic activity under these test conditions (REACHa).

In another in vitro study, the oestrogenic activity of methylparaben was assessed using the yeast two-hybrid assay, incorporating oestrogen receptors and using a human oestrogen receptor enzyme-linked immunosorbent assay. Methylparaben had no detectable oestrogenic activity in either assay (REACHa).

The oestrogenic activity of methylparaben was assessed in a competitive binding assay using MCF-7 human breast cancer cells. The test chemical bound to oestrogen receptors with low affinity (requiring a minimum concentration of 500,000-fold molar excess over 17β-estradiol) and gave a very weak effect on oestrogen-associated cell proliferation. On the basis of these findings, the test chemical was determined to exhibit very low oestrogenic activity (REACHa).

A competitive binding assay was conducted to examine the oestrogenic activity of methylparaben. Uteri from ovariectomised SD rats were homogenised and used to generate an oestrogen receptor-rich suspension for the assay. The chemical exhibited weak binding to the oestrogen receptor (relative binding affinity of 0.0004 % of the positive control—17β-estradiol). The

calculated IC50 (50 % inhibition of the 17β-estradiol binding) was 0.25 mM compared with an IC50 of 0.9 nM for 17β-estradiol. The data indicated that methylparaben possessed weak oestrogenic activity (REACHa).

In an in vitro investigation, MCF-7 human breast cancer cells were incubated with methylparaben for seven days at 0.5 mM. A human expression microarray system was then used to profile the expression of approximately 20,000 genes in the cells. MCF-7 cell growth and viability were also assessed. The test chemical was found to increase cell proliferation; however, the pattern of gene expression differed significantly from that promoted by the positive control 17β-estradiol.

The oestrogenic activity of ethylparaben was assessed in an in vivo study conducted in a similar fashion to OECD TG 440 (uterotrophic bioassay in rodents). Female B6D2F1 mice were exposed to the test chemical daily for three days at 100 mg/kg bw/day. The chemical failed to cause a statistically significant increase in uterine weights and, on the basis of this result, the oestrogenic activity of ethylparaben under these conditions was found to be negligible (REACHb).

Ethylparaben was assessed for its endocrine activity in immature female SD rats. Reproductive and fertility endpoints were also assessed. Animals were dosed at 62.5, 250 or 1000 mg/kg bw/day by oral gavage for 20 days. No significant effect on the oestrus cycle was observed at any dose. No significant change in uterine or ovarian weights was observed in animals in any dose group. Histopathological assessment revealed no significant change in the uteri of the exposed animals. A decrease in the number of corpora lutea was observed in the exposed animals' ovarian follicles (REACHb)

In an in vivo study, the potential for ethylparaben to affect oestrogenic function was investigated. Developmental toxicity was also assessed in this study. Pregnant Wistar rats were dosed with the chemical via subcutaneous injection at 400 mg/kg bw/day from GD 7–21. No statistically significant effects on body weight, foetal or maternal toxicity were observed, except for one dam that had 5 % late resorptions. No statistical or biologically significant effects were observed in any hormone plasma concentration. The test chemical also did not affect the expression of a number of genes associated with oestrogenic activity. Reproductive organs from foetuses were histologically similar to controls (REACHb).

Ethylparaben was assessed for oestrogenic activity in a study conducted according to OECD TG 440. Female Wistar rats were administered the chemical via subcutaneous injections at 6, 18, 60 and 180 mg/kg bw/day for three days. In the highest dose group, a significant increase in uterine weights was observed, indicating some oestrogenic activity (REACHb).

In another OECD TG 440 study, ethylparaben was administered to female B6D2F1 mice by oral gavage at 1000 mg/kg bw/day for three consecutive days. The test substance did not influence uterus wet weights or the uterus to body weight ratio (REACHa).

In a cell proliferation and competitive binding study, ethylparaben was shown to have oestrogenic activity when incubated with human MCF-7 human breast cancer cells. This activity was completely inhibited by a pure oestrogen antagonist. However, the chemical was considered to be a weakly oestrogenic, as it induced only a low proliferative effect when compared with the positive control 17β-estradiol. The investigation showed that the test substance binds with weak affinity to ERa and ERβ (relative binding affinity of 0.011 % compared with 17β-estradiol) (REACHb).

The oestrogen receptor binding potential of propylparaben was studied in vitro, using oestrogen receptors harvested from excised uteri. The binding affinity of the test chemical was compared with 17β-estradiol. The chemical bound with low affinity to ERs (with a relative binding affinity of 0.0004 % when compared with 17β-estradiol) (REACHc).

Propylparaben was assessed for oestrogenic activity in a study conducted in compliance with OCED TG 440. Immature female B6D2F1 mice were administered the chemical via subcutaneous injections at 100 mg/kg bw/day for three days. No statistically significant effects were observed in response to treatment with propylparaben (REACHc).

Propylparaben was assessed for its oestrogenic activity in female SD rats. Reproductive and fertility endpoints were also assessed. Animals were dosed with the chemical by oral gavage at 62.5, 250 or 1000 mg/kg bw/day for 20 days. No significant effect on the oestrus cycle was observed at any dose. Some histopathological changes were observed in the uteri of animals in the highest dose group (including myometrial hypertrophy and uterine wall-thickening). There was also a decrease in the number of corpora lutea in ovarian follicles in all three treatment groups (REACHc).

Propylparaben was investigated for oestrogenic activity in a study conducted according to OCED TG 440. Female CD-1 mice (ovariectomised and immature) were dosed with the test chemical at 0.65, 6.5, 20, 65 or 195 mg/kg bw/day, for immature mice and at 6.5, 20, 65 or 195 mg/kg bw/day for ovariectomised mice, on consecutive days for a period of three days. Significant increases in uterine weights were observed in immature mice in the 20, 65 and 195 mg/kg bw/day groups. Increases in uterine

weights were also observed in ovariectomised mice in the 20, 65 and 195 mg/kg bw/day groups. Several histopathological abnormalities were identified in the two highest dose ovariectomised groups (no other groups were assessed histopathologically). These changes included increased luminal epithelial height, glandular epithelial height and myometrial width in the uteri. On the basis of these effects, the test chemical was determined to have oestrogenic activity (REACHc).

Butylparaben has been shown to compete with 3H-estradiol for binding to rat oestrogen receptors in a competitive binding assay. The chemical exhibited a reported relative binding affinity of 0.0009 % in comparison with 17β-estradiol. In vivo investigations have demonstrated that butylparaben produces a positive uterotrophic response in rats when administered via subcutaneous injection (Danish EPA, 2001).

Isobutylparaben exhibited significant oestrogenic activity in a mouse uterotrophic assay where animals were administered the chemical via subcutaneous injection once daily for three days at 1.2 or 12 mg/animal. This activity was very low when compared with estradiol (Anderson, 2008).

Oestrogenic activities of the parabens are thought to increase relative to the length and branching of the alkyl ester (Darbre et al., 2004).

Risk Characterisation

Critical Health Effects

No critical health effects associated with these chemicals have been established, although they do have very weak oestrogenic activity.

Public Risk Characterisation

Considering the range of domestic, cosmetic and personal care products that could contain the chemicals, the main route of public exposure is expected to be through the skin, inhaled from products applied as aerosols, and potential oral exposure from lip and oral hygiene products.

The available data do not indicate any risks associated with exposure to the chemicals in this group. The chemicals have been shown to have weak oestrogenic activity, but there are no established adverse outcome pathways for weak oestrogenic activity. Should further information on adverse outcome pathways in mammals associated with weak oestrogenic activity become available, further assessment of these chemicals at Tier III may be required.

Occupational Risk Characterisation

During product formulation, dermal, oral and ocular exposure might occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemicals at lower concentrations could also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of application and work practices employed.

Given the critical health effects, the risk to workers from this chemical is not considered to be unreasonable. The chemical currently has no hazard classification for worker health and safety; this is considered appropriate based on the available data.

NICNAS Recommendation

Current risk management measures are considered adequate to protect public and workers' health and safety, provided that all requirements are met under workplace health and safety, and poisons legislation as adopted by the relevant state or territory.

The available data do not indicate any risks associated with exposure to the chemicals in this group. The chemicals have been shown to have weak oestrogenic activity, but there are no established adverse outcome pathways for this effect. Should further

information on adverse outcome pathways in mammals associated with weak oestrogenic activity become available, further assessment of these chemicals at Tier III could be required.

Regulatory Control

Advice for consumers

Products containing the chemicals should be used according to the instructions on the label.

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Last Update 24 April 2015

Chemical Identities

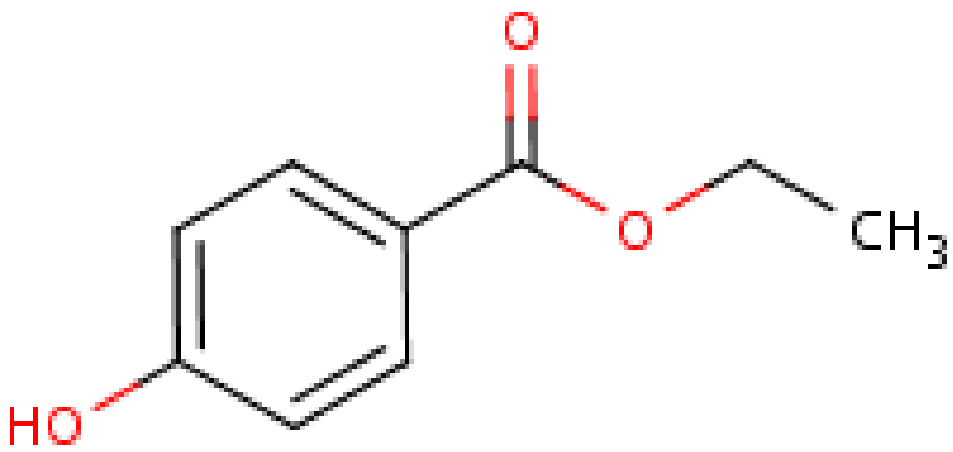
Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, propyl ester** propylparaben propyl parahydroxybenzoate 4-hydroxybenzoic acid, propyl ester
CAS Number	94-13-3
Structural Formula	
Molecular Formula	C10H12O3

Molecular Weight	180.202

Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, butyl ester** butyl p-hydroxybenzoate butylparaben 4-hydroxybenzoic acid, butyl ester butyl parahydroxybenzoate
CAS Number	94-26-8
Structural Formula	
Molecular Formula	C11H14O3
Molecular Weight	194.2286

Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, methyl ester** methyl p-hydroxybenzoate methylparaben 4-hydroxybenzoic acid, methyl ester methyl parahydroxybenzoate
CAS Number	99-76-3
Structural Formula	
Molecular Formula	C8H8O3
Molecular Weight	152.1482

Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, ethyl ester** ethyl p-hydroxybenzoate ethylparaben 4-hydroxybenzoic acid, ethyl ester ethyl parahydroxybenzoate
CAS Number	120-47-8
Structural Formula	

	
Molecular Formula	C9H10O3
Molecular Weight	166.175

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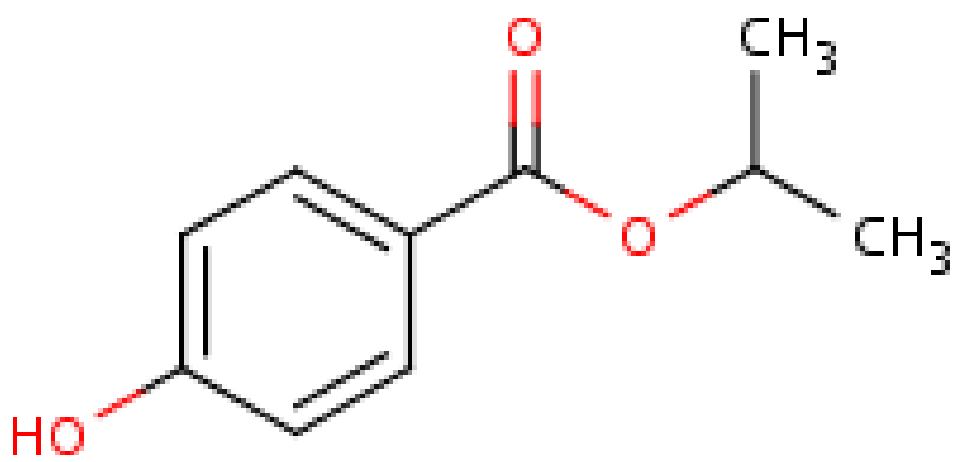
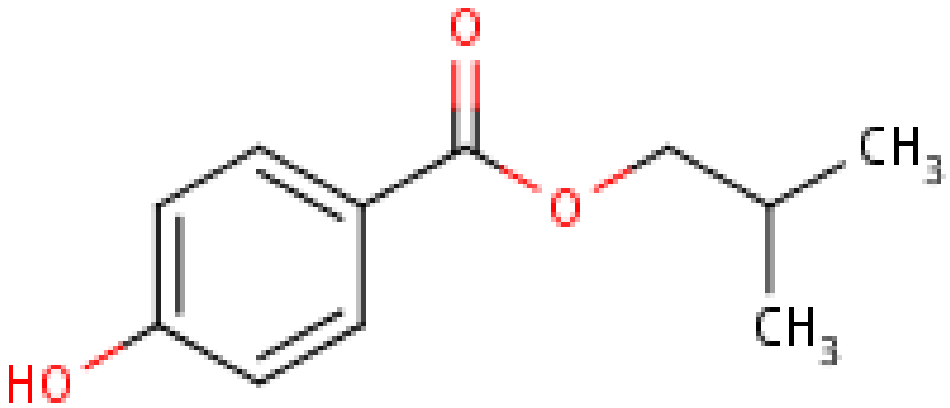
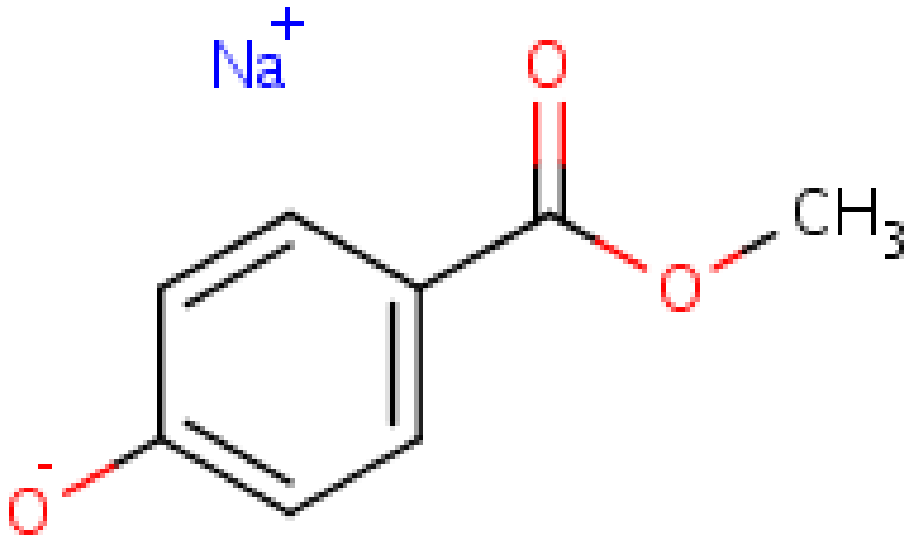
CAS Number	4191-73-5
Structural Formula	

	
Molecular Formula	C10H12O3
Molecular Weight	180.202

Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, 2-methylpropyl ester** isobutyl p-hydroxybenzoate isobutylparaben isobutyl-4-hydroxybenzoate benzoic acid, 4-hydroxy-, 2-methylpropyl ester
CAS Number	4247-02-3
Structural Formula	

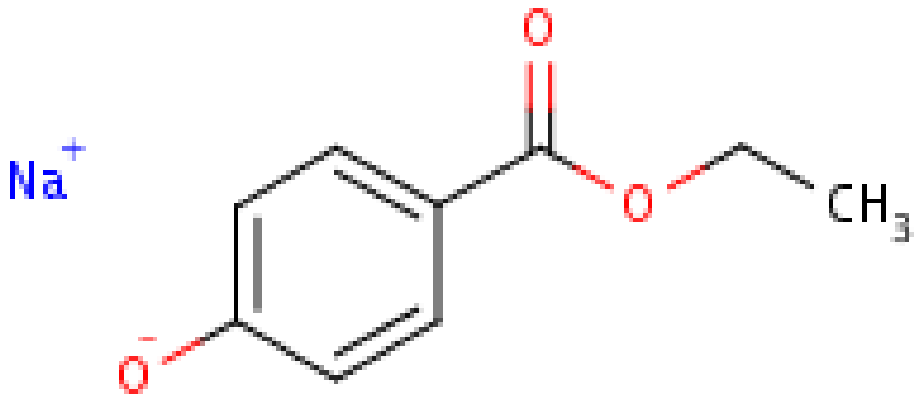
	
Molecular Formula	C11H14O3
Molecular Weight	194.229

Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, methyl ester, sodium salt** sodium methyl hydroxybenzoate sodium methylparaben benzoic acid, 4-hydroxy-, methyl ester, sodium salt methyl p-hydroxybenzoate, sodium salt
CAS Number	5026-62-0
Structural Formula	

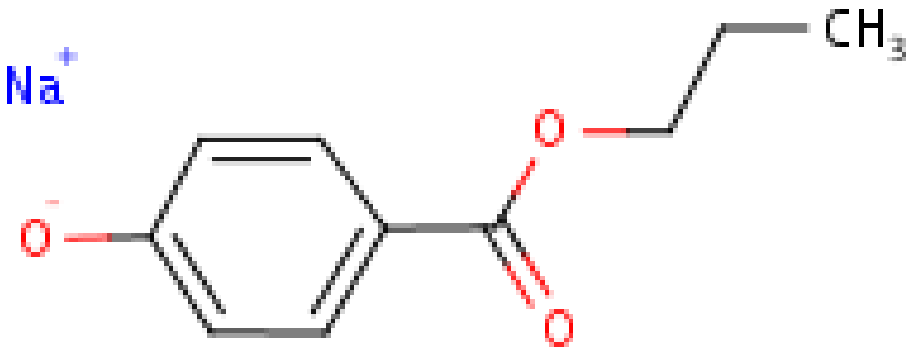
	
Molecular Formula	C8H8O3.Na
Molecular Weight	174.1303

Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, methyl ester, potassium salt** methyl p-hydroxybenzoate, potassium salt potassium methylparaben benzoic acid, 4-hydroxy-, methyl ester, potassium salt 4-hydroxybenzoic acid, methyl ester, potassium salt
CAS Number	26112-07-2
Structural Formula	
Molecular Formula	C8H8O3.K
Molecular Weight	190.2383

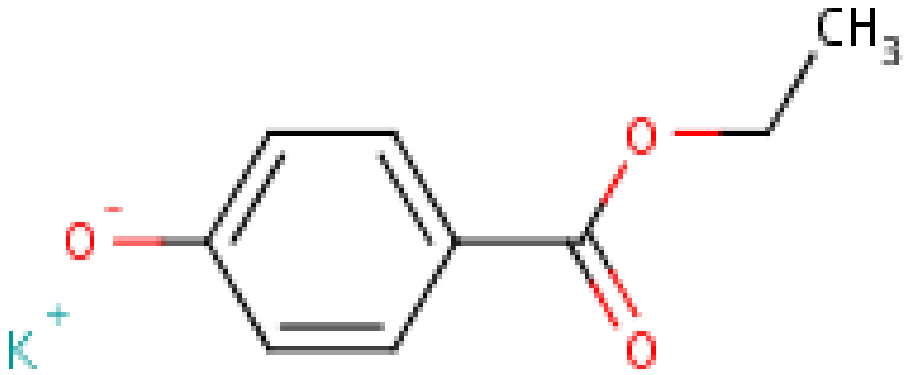
Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, ethyl ester, sodium salt** sodium ethyl hydroxybenzoate 4-hydroxybenzoic acid, ethyl ester, sodium salt

	ethylparaben sodium ethyl p-hydroxybenzoate, sodium salt sodium ethylparaben
CAS Number	35285-68-8
Structural Formula	
Molecular Formula	C9H10O3.Na
Molecular Weight	188.157

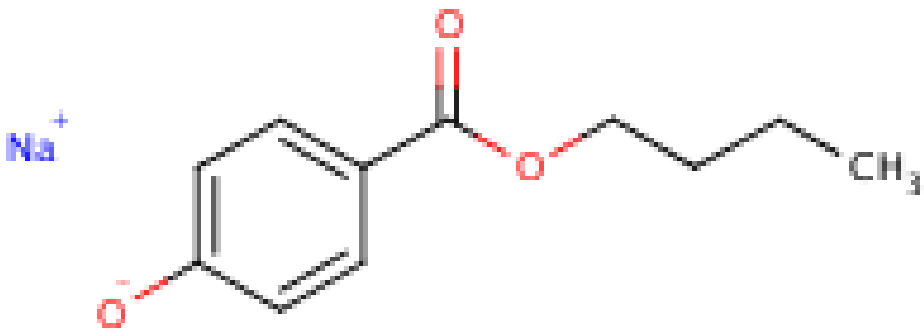
Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, propyl ester, sodium salt** propyl 4-hydroxybenzoate, sodium salt sodium propylparaben propylparaben, sodium salt 4-hydroxybenzoic acid, propyl ester, sodium salt
CAS Number	35285-69-9
Structural Formula	

	
Molecular Formula	C10H12O3.Na
Molecular Weight	202.184

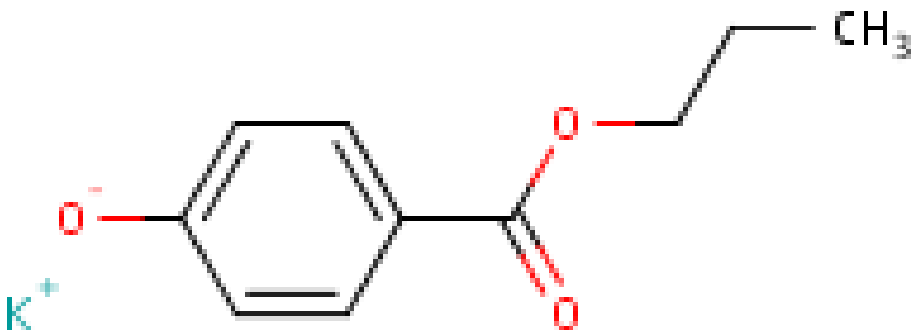
Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, ethyl ester, potassium salt** benzoic acid, p-hydroxy-, ethyl ester, potassium salt potassium ethyl 4-oxidobenzoate potassium ethylparaben
CAS Number	36457-19-9
Structural Formula	

	
Molecular Formula	C9H10O3.K
Molecular Weight	204.2651

Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, butyl ester, sodium salt** butyl 4-hydroxybenzoate, sodium salt sodium butyl 4-hydroxybenzoate butylparaben sodium sodium butyl hydroxybenzoate benzoic acid, p-hydroxy-, butyl ester, sodium salt
CAS Number	36457-20-2
Structural Formula	

	
Molecular Formula	C11H14O3.Na
Molecular Weight	216.211

Chemical Name in the Inventory and Synonyms	**Benzoic acid, 4-hydroxy-, propyl ester, potassium salt** potassium propyl 4-oxidobenzoate potassium propylparaben
CAS Number	84930-16-5
Structural Formula	

	
Molecular Formula	C10H12O3.K
Molecular Weight	218.2919

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