Fluorescein and its sodium salt: Human health tier II assessment

02 March 2018

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy-, disodium salt	518-47-8
Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy-	2321-07-5

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**

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemicals in this group are fluorescein (CAS No. 2321-07-5) and its soluble sodium salt, sodium fluorescein (CAS No. 518-47-8). These chemicals have similar uses in a wide variety of products or applications as indicators and dyes (see **Use** section). The counter ion is not expected to drive toxicity (NICNAS).

The chemicals have a distinctive orange/red colouring as solids and the sodium salt is soluble in water with a strong yellow colour allowing it to be used as a dye within products, medicines, and as a water tracing agent.

Import, Manufacture and Use

Australian

No specific industrial Australian use, import, or manufacturing information has been identified for fluorescein (CAS No. 2321-07-5).

The following non-industrial uses for the fluorescein, sodium salt (CAS No. 518-47-8) have been identified in Australia (Therapeutic Goods Administration (TGA)):

- as an intravenous solution for fluorescein angiography;
- as numbing eye drops for eye examinations; and
- as fluorescein eye strips to stain the eye for examination, diagnosis, and fitting contact lenses.

The chemical fluorescein, sodium salt (CAS No. 518-47-8) has reported cosmetic use in toothpaste (PlaqPro) at 0.25 % as a plaque indicator. The chemical also has reported commercial and site-limited use as a generic 'dye'.

International

The following international uses have been identified through the European Union (EU) Registration, Evaluation, Authorisation and Restriction 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 Products Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary; US Department of Health and Human Services, Household Products Database (HPD) and; the US National Library of Medicine's Hazardous Substances Data Bank (HSDB).

The chemicals have reported cosmetic uses as colourants in personal care products:

- fluorescein, sodium salt (CAS No. 518-47-8) in soaps, cleaners, bath gels, shaving cream; and
- fluorescein (CAS No. 2321-07-5) in blushers, cleaners, colognes, hair preparations (non-colouring), nail polish, shampoos (non-colouring).

The chemicals have reported commercial uses, including as:

- a marker for sea rescues;
- an aquatic dye for tracing water courses and water leaks; and
- a colourant in laminates and antifreeze.

The chemical fluorescein, sodium salt (CAS No. 518-47-8) has reported non-industrial uses, including:

- as an intravenous solution for fluorescein angiography; and
- as numbing eye drops for eye examinations; and
- as eye strips to stain the eye for examination, diagnosis, and fitting contact lenses.

Restrictions

Australian

These chemicals are listed in the *Poisons Standard—the Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP) in Schedule 4 under 'FLUORESCEIN in preparations for injection' (SUSMP, 2018).

Schedule 4 chemicals are described as 'Substances, the use or supply of which should be by or on the order of persons permitted by State or Territory legislation to prescribe and should be available from a pharmacist on prescription'.

International

Fluorescein (CAS No. 2321-07-5) is listed on the following (Galleria Chemica):

- ASEAN Cosmetic Directive Annex II Part 1: List of substances which must not form part of the composition of cosmetic products;
- EU Regulation (EC) No 1223/2009 - Annex II - List of Substances Prohibited in Cosmetic Products (Fluorescein and its disodium salt (Acid Yellow 73 sodium salt; CI 45350) when used as a substance in hair dye products);
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain;

- EU Commission List of substances banned for use in hair dye products.

Fluorescein sodium salt (CAS No. 518-57-8) is listed on the following (Galleria Chemica):

- ASEAN Cosmetic Directive Annex II Part 1: List of substances which must not form part of the composition of cosmetic products;
- ASEAN Cosmetic Directive ANNEX IV - Part 1- List of colouring agents allowed for use in cosmetic products, at a maximum of 6% concentration in the finished product (Yellow 45350);
- EU Regulation (EC) No 1223/2009 - Annex II - List of Substances Prohibited in Cosmetic Products (Fluorescein and its disodium salt (Acid Yellow 73 sodium salt; CI 45350) when used as a substance in hair dye products);
- EU Regulation (EC) No 1223/2009 - Annex IV - List of Colourants allowed in cosmetic products, at up to 6% concentration (Yellow 45350);
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain;
- New Zealand Cosmetic Products Group Standard—Schedule 6: Colouring Agents Cosmetic Products May Contain With Restrictions, up to 6% maximum concentration in the finished product (Yellow 45350);
- EU Commission List of substances banned for use in hair dye products.

Existing Worker Health and Safety Controls

Hazard Classification

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

Exposure Standards

Australian

No specific exposure standards are available.

International

The following exposure standards are identified for the chemicals (Galleria Chemica).

Temporary Emergency Exposure Limits (TEELs) defined by the US Department of Energy for fluorescein:

- TEEL-1 = 0.78 mg/m^3;
- TEEL-2 = 8.6 mg/m^3; and
- TEEL-3 = 65 mg/m^3.

TEELs defined by the US Department of Energy for fluorescein sodium salt:

- TEEL-1 = 20 mg/m^3;
- TEEL-2 = 220 mg/m^3; and
- TEEL-3 = 1300 mg/m^3.

Health Hazard Information

The chemicals fluoresce green or yellow under blue light and this property is used for water tracing applications and eye angiography. Most of the data available are for fluorescein sodium salt. Therefore, unless specifically stated, the test substance used in the studies below is fluorescein sodium salt and is referred to as 'the chemical'.

Toxicokinetics

The chemical has therapeutic use in human eye angiography; therefore, there are data available on the toxicokinetics in humans.

Available toxicokinetic studies of the chemical in humans are through the intravenous route. Following administration, the chemical is rapidly distributed throughout the body. The chemical binds to albumin and red blood cells reversibly and yellowish discolouration of the skin occurs within a few minutes of injection, which fades over 24 hours. Notably, fluorescein distributes to retinal tissues and the central artery of the eye within 7 to 14 seconds (HSDB; Pubchem).

Fluorescein is metabolised rapidly into fluorescein monoglucuronide (~80 % in plasma converted to the conjugate after a 1 hour period). When the plasma was measured in a fluorophotometer, fluorescein monoglucuronide was observed to have 1/4–1/3 of the fluorescence intensity of fluorescein, and has a half-life of approximately 264 minutes compared with fluorescein at 23.5 minutes. Therefore, the glucuronide conjugate contributes to almost all plasma fluorescence after 4–5 hours (HSDB; Pubchem).

Fluorescein and its metabolites are mainly eliminated via renal excretion. Following administration, the urine remains slightly fluorescent for 24–36 hours. Systemic clearance of fluorescein is completed by 48–72 hours at a dose of 500 mg. When used in nursing mothers, fluorescein has been demonstrated to be excreted in human milk for up to 4 days (HSDB; Pubchem).

Acute Toxicity

Oral

Based on the available data, the chemical is considered to have low acute toxicity following oral exposure.

Reported median lethal doses (LD50) were 4470–4738 mg/kg bw in mice and 4738–6720 mg/kg bw in rats. Reported clinical signs in mice included ataxia, death, central nervous system depression and decreased spontaneous motor activity (REACH).

Dermal

No data are available.

Inhalation

No data are available.

Observation in humans

A study was conducted in Japan to check eye abnormalities in humans following oral ingestion of the chemical. Over 8 years, a total of 1787 patients were treated with a dose of 10 mL at 10 % concentration and observed. No adverse effects were reported. A LD0 of 2,500 mg/kg bw was reported. Side effects were observed in 3 % (8 of 266) people who underwent injection fluorescein angiography; including itching, discomfort and nausea. The patients recovered after a 1-hour rest period (REACH).

Corrosion / Irritation

Skin Irritation

Only limited information is available.

The chemical is reported to be non-irritating in a study conducted on mammals. No other details are available (REACH). This is supported by Quantitative Structure-Activity Relationships (QSAR) predictions using OECD toolbox version 3.3 on New Zealand White rabbits (REACH).

Eye Irritation

Based on the available data, slight to moderate irritation was observed in rabbits at a concentration of 2 % of the chemical on repeated exposure. However, the available information is not sufficient to warrant hazard classification.

In an eye staining study, the chemical (0.1 mL at a 2 % aqueous solution) was instilled in the lower conjunctival sac of each eye of 4 groups of New Zealand White rabbits (n = 3/sex/group). The duration of exposure was 15 sec, 30 sec, 1 minute or 4 minutes, daily for 10 days over 2 weeks. The treated eyes were flushed out with saline solution. The eyes were scored based on the degree of staining of the eye, and the mean irritation score over all 4 timepoints was 1–2 (slight to moderate). Effects were reversible within 48 hours. The chemical was stated to be mild to moderately irritating (REACH).

Eye irritation potential for the chemical was estimated using OECD QSAR Toolbox 3.4 and was estimated to be not irritating to the eyes of New Zealand White rabbits (REACH).

In a Draize eye irritation study in rabbits, fluorescein was found to be highly irritating with severe reactions observed at a 100 µL / 24 hr dose. No other details are available (RTECS).

Observation in humans

The chemical is reported to be an eye, skin and/or a respiratory tract irritant (HSDB). However, the majority of the studies available are due to its therapeutic use in eye angiography and through intravenous administration.

The chemical is regularly used in eye angiography studies at a dose of 500 mg up to a concentration of 25 % in solution (HSDB; Pubchem).

In a human patch test study, the chemical was applied undiluted and at 1:10 dilution in sterile water to a 65 year old patient under occlusion for 2 days, with observation up to 4 days. Positive reactions (violaceous bullous lesions) were observed on day 3. The chemical was reported to be a skin irritant according to the International Contact Dermatitis Research Group criteria (REACH).

Sensitisation

Skin Sensitisation

There was no information available on the sensitisation potential of the chemical in animals. Based on available human data, the chemical is not considered to be a skin sensitiser (see **Observation in humans**).

Observation in humans

Skin prick and intradermal tests were conducted on 1037 patients using increasing concentrations of the chemical at 1, 10 or 100 % for the skin prick tests, and 10 % for intradermal tests. No positive results were recorded and the chemical was considered non-sensitising (REACH).

In an intradermal skin test conducted on 196 male and female patients, the chemical was administered at 0.2 mL at 2 % concentration. Positive reactions were observed in 12 of the 196 patients (6 %) (REACH).

In a patch test study conducted on 16 patients, only 1 positive reaction was reported following application of the chemical at 1 % concentration in petrolatum (REACH).

There are multiple reported cases of anaphylactic shock induced by eye drops (containing the chemical at 2 % concentration) in human patients of both genders (REACH).

In 2 case studies, 2 patients (a 23-year old woman and a 75 year-old man) reacted positively to skin prick tests at 0.2 % concentration of the chemical (REACH).

In another patch test conducted on one male patient, the chemical was not a skin sensitiser when tested at 20 % concentration alongside positive and negative controls of histamine and saline solutions (REACH).

Repeated Dose Toxicity

Oral

Based on the available data, the chemical is not considered to cause damage to health from repeated oral exposure.

In a 14-day combined repeat dose and reproductive/developmental toxicity study (OECD test guideline (TG) 422), female Sprague-Dawley rats (n=25/dose) were administered the chemical at 0, 100, 500 and 1500 mg/kg bw/day by oral gavage. Mortality and discolouration of small intestines were observed at the highest dose, as well as discolouration of urine and amniotic fluid at dose levels of 100 mg/kg bw and above. A No Observed Adverse Effect Level (NOAEL) of 500 mg/kg bw/day was established (REACH).

In a 14-day repeat dose oral toxicity study (OECD TG 422), Dutch belted female rabbits (n=14/dose) were administered the sodium salt at 0, 30, 100 and 250 mg/kg bw/day by oral gavage. There was one mortality in each of the 30, 250 mg/kg bw/day and control groups. Pneumonia was observed in all three rabbits following necropsy. No effects were observed on body weight, body weight gain and gross pathology compared to the control. Therefore the NOAEL was considered to be 250 mg/kg bw/day for the chemical in this study (REACH).

Dermal

No data are available.

Inhalation

No data are available.

Genotoxicity

Based on the available information the chemical is not expected to be genotoxic.

The chemical gave largely negative results in several in vitro genotoxicity assays (REACH):

- negative in several bacterial reverse mutation assays with strains of *Salmonella typhimurium* and *Escherichia coli*, with or without metabolic activation;

- negative in chromosomal aberration tests in mammalian cell cultures and Chinese hamster ovary (CHO) cells, with and without metabolic activation;
- negative in microsome assays in *S. typhimurium* and mammalian cells, with and without metabolic activation.
- negative in mouse lymphoma cells without metabolic activation, but positive with metabolic activation.
- positive in sister chromatid exchange in CHO cells (NTP); and
- positive at the TK locus in L5178Y mouse lymphoma cells in male mice (NTP).

The chemical gave negative in vivo results for reciprocal translocation or sex-linked recessive lethal mutagenesis in *Drosophilia*, and in a bone marrow micronucleus assay in male mice (NTP).

The chemical gave negative in silico modelling results in Chinese hamster lung fibroblast and CHO fibroblast cells (NTP).

Carcinogenicity

No data are available.

Reproductive and Developmental Toxicity

Based on the limited information available the chemical is not considered to be toxic to reproduction or development.

In a one-generation reproductive toxicity study (OECD TG 421), a group of New Zealand White female rabbits were administered the chemical at 2240 mg/kg bw/day intravenously at 10 % in aqueous solution at days 5, 6, 8, 13, 15 and 16 after copulation. No effects on oestrous cycles, birth defects, soft tissues, visceral abnormalities or pathological changes were observed throughout the experiment. One stillbirth occurred but all other offspring were normal. A total of 14 offspring from 4 mothers died at week 4, this was attributed to poor health of the mothers and not to the treatment. The NOAEL was considered to be 2240 mg/kg bw/day for F0 and F1 generations (REACH).

In a one-generation reproductive toxicity study (OECD TG 421) a group of Dutch-Belted female rabbits were administered the chemical by oral gavage up to 250 mg/kg bw/day between days 6 and 27 of gestation. Malformations were observed at 30 and 100 mg/kg bw/day in 2 foetuses from 2 litters; however these values fell within the ranges of historical control data. No biological or statistically significant differences in the number of foetuses with malformations and the number of foetuses or litters with developmental variations were observed. The NOAEL was considered to be 250 mg/kg bw/day for F0 and F1 generations (REACH).

In a combined reproductive and developmental toxicity study (OECD TG 421) a group of Sprague Dawley female rats (n=25/group) were administered the chemical by oral gavage at 0, 100, 500 or 1500 mg/kg bw for 14 days. When treated with 1500 mg/kg bw/day 6 rats died. No significant effects on reproduction and development were observed on body weight, number of litters, foetal sex ratio, post-implantation loss/dam, total implantations/dam and corpora lutea/dam. The NOAEL was considered to be 1500 mg/kg bw/day for F0 and F1 generations (REACH).

Risk Characterisation

Critical Health Effects

No critical health effects for risk characterisation were identified for these chemicals. While no skin sensitisation was reported in the largest study group in humans, positive results were reported in several smaller groups and case studies. There are also reported cases of anaphylaxis; therefore, care should be taken when using these chemicals.

Public Risk Characterisation

Fluorescein, sodium salt (CAS No. 518-47-8) has cosmetic use in toothpaste at 0.25 % concentration in Australia. Although other uses in cosmetic and domestic products in Australia are not known, fluorescein, sodium salt is approved for use in cosmetic products (except hair dyes) overseas at concentrations up to 6 % in the finished product. Given the low hazard of the chemicals, the chemicals are not considered to pose an unreasonable risk to public health.

The chemicals are currently listed on Schedule 4 of the SUSMP under 'FLUORESCEIN' in preparations for injection. Use of the chemical in therapeutic products is regulated by the Theraputic Goods Administration (TGA).

Occupational Risk Characterisation

During product formulation, 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.

Based on the available data, the lack of hazard classification in the HCIS (Safe Work Australia) is considered appropriate.

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. No further assessment is required.

Should any information become available to indicate significant consumer exposure to the chemicals to cosmetic and domestic products in Australia, this assessment may be revisited.

Regulatory Control

Public Health

Although the chemicals are listed under 'FLUORESCEIN' in Schedule 4 for medicines, no further restrictions are required for industrial uses.

Work Health and Safety

The chemicals are not recommended for classification and labelling aligned with the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). This does not consider classification of physical hazards and environmental hazards.

Advice for consumers

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

Advice for industry

Control measures

Control measures to minimise the risk from ocular exposure to the chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemicals are used. Examples of control measures that could minimise the risk include, but are not limited to:

- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemicals.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemicals are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of these chemicals has not been undertaken as part of this assessment.

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information/imap-assessments/how-chemicals-are-assessed/identification-of-chemicals-of-low-concern-to-human-health

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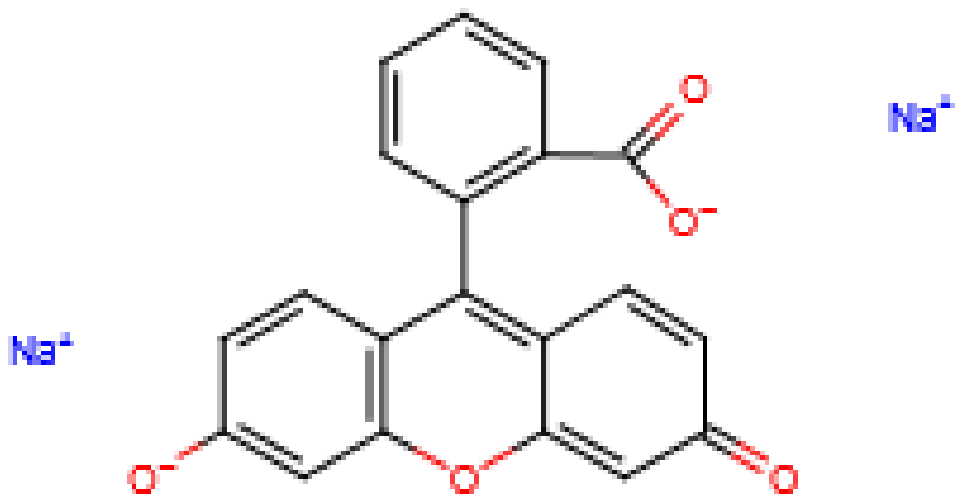
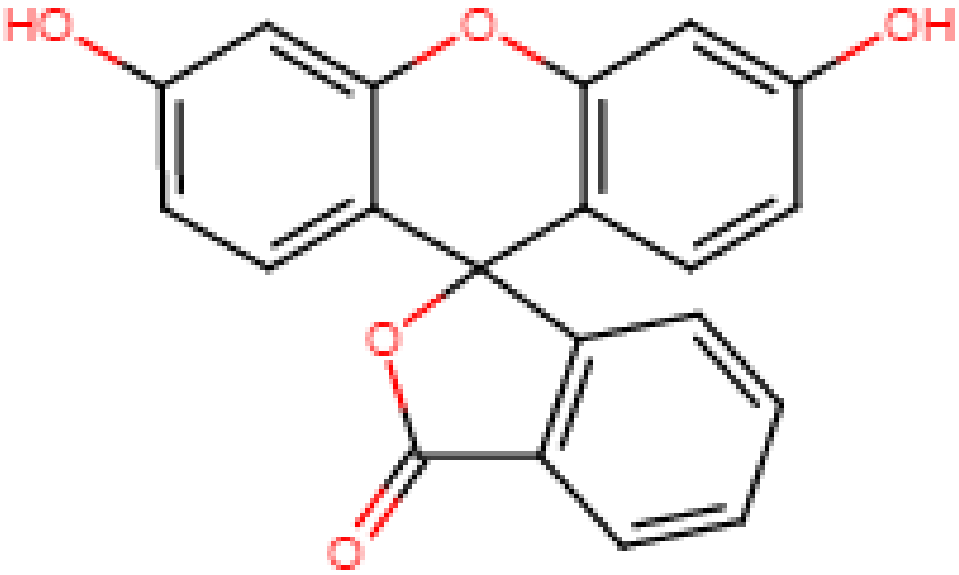
Last Update 02 March 2018

Chemical Identities

Chemical Name in the Inventory and Synonyms	**Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy-, disodium salt** C.I. Acid Yellow 73 fluorescein, sodium salt Yellow 45350 CI 45350 Ki202(1) or Ki202(2)
CAS Number	518-47-8
Structural Formula	

	
Molecular Formula	C20H12O5.2Na
Molecular Weight	376.274

Chemical Name in the Inventory and Synonyms	**Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 3',6'-dihydroxy-** fluorescein
CAS Number	2321-07-5
Structural Formula	

	
Molecular Formula	C20H12O5
Molecular Weight	332.3098

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