Final Report

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1. Inception Phase

1.1 Background

The field of predictive toxicology has an urgent need for the development of open, public, computable standardized toxicology vocabularies and ontologies to support the applications required by *in silico, in vitro* and *in vivo* toxicology methods, and by related reporting activities such as the REACH [1] (Registration Evaluation and Authorization of Chemicals) legislation.

All predictive approaches in toxicology actually share the need of highly structured information as a starting point. The definition of ontology and of controlled vocabulary is a crucial requirement in order to: a) standardize and organize the chemical and toxicological databases on which the predictive toxicology methods build on; b) improve the interoperability between toxicology resources; and c) create a knowledge infrastructure supporting R&D and risk assessment.

The goal of the Project is to develop ontologies suitable for standardizing and organizing the chemical toxicological databases in the OECD (Q)SAR Toolbox [2]. Three ontologies (skin irritation, eye irritation, skin and respiratory sensitisation) are to be developed, each with a targeted work period of 3 months.

The Open Biomedical Ontologies Foundry [3] principles, the Web Ontology Language (OWL) and the Protégé software will be used for the implementation.

This Deliverable Report describes all related resources that have been identified during the Inception Phase of the Project. A number of different tasks and subtasks have been identified. Detailed description of the tasks associated with each of the three work packages (WP) (one for each endpoint), as well as acceptance and quality insurance plans are also provided. The total time allocated for completing the project is twelve months.

1.2 Review of existing work: Deliverable 1.1

1.2.1 Review of database included in the OECD (Q)SAR Toolbox version 3.

Datasets available in the OECD (Q)SAR Toolbox version 3.0.

The goal of the Project is develop of ontologies suitable for standardizing and organizing the chemical toxicological databases in the OECD (Q)SAR Toolbox for three toxicological endpoints: skin irritation, eye irritation, skin and respiratory sensitization (see Table 1 for list of datasets to be covered by ontology as to Annex 5 SC2).

A first requirement is obviously the compatibility of the Ontology with the existing terminology used in the OECD (Q)SAR Toolbox and the flexible ontology approach suitable for further possible extensions.

Endpoint	Database name	Assay	Number of chemical	Number of data	Reference Donator
Skin sensitisation	Skin sensitisation	LLNA	411	411	Uniliver; Contact Dermatitis; Givaudan; J. Med. Chem., 51, Regulatory Toxicology and Pharmacology; Springer-Verlag Berlin Heidelberg Toxicological sciences journal; Toxicology in vitro journal
		GPMT	276	276	SAR QSAR Environ. Res.; International Journal of Toxicology; Contact Dermatitis; Arch Dermatol Res.
		BfR	178	178	Medizin&Wissen Verlagges, Munchen
		Human data	1	1	Contact Dermatitis
		Animal data*	10	10	RIFM
		Undefined assay	213	568	UMU, UK
	Skin sensitization ECETOC	Undefined assay	31	31	TR77 Skin and Respiratory Sensitisers - Reference Chemicals Data Bank.pdf
Skin sensitization (Adverse outcome pathways)**	Dendritic celi activity	Dendritic Celi Activity (h-CLAT)	100	200	Dr. Schultz
		Dendritic Celi Activity (MUSST)	42	44	Dr.Schultz
	Skin sensitization AOP	LLNA	137	123	Dr.Schultz
	Keratinocyte gene expression	Keratinocyte Gene Expression (ARE)	100	298	Dr.Schultz
	Chemical reactivity	% Depletion of Cystine	104	106	Dr. Schultz
		% Depletion of Lysine	103	105	
		Adduct Formation	72	74	
	GSH reactivitv	GSH RC 50	424	1022	Dr.Schultz
Skin irritation/ corrosion	Skin irritation corrosion	Primary Irritation index	354	558	National Institute for Public Health and the Environment (RIVM)
Eye irritation/corrosion	Eye irritation ECETOC	MMAS	128	146	National Institute for Public Health and the Environment (RIVM)

* Animal data – Animal Data Using Classification Defined in ECETOC, Technical Report No. 87, 2003

** Skin sensitization (Adverse outcome pathways) databases related are available in upcoming version QSAR Toolbox 3.0

Table 1. Datasets to be covered by ontology (Annex 5 SC2)

Currently the OECD (Q)SAR Toolbox version 3.0 [2] contains generation of the following databases (DBs) relative to the endpoints to be covered by ontology, as required by the Specific Contract 2 (see Table 2 for more detailed information):

-skin sensitization;

-skin sensitization ECETOC;

-dendritic cells COLIPA;

- keratinocyte gene expression Givaudan;

- chemical Reactivity COLIPA;

- GSH Experimental RC50;

- skin irritation;

- eye Irritation ECETOC.

In sections 2.1.2-2.1.9 we will review all databases listed below. Some small differences in datasets characteristics (number of data, donators name, etc) in Table 1 and Table 2 are due to the fact that Table 2 is based on the latest search within version 3.0 of the QSAR Toolbox while the Table 1 has been prepared before the v. 3.0 release.

Database name	Donators	Number of chemicals	Number of data	Number of endpoints	Name of endpoints
Skin sensitisation	Unilever; Procter & Gamble; ExxonMobil; Organization for Economic Co-operation and Development (OECD)	1035	1570 (437-LNNA assay; 330-GMTP assay; 236- BfR list; 567- LJMU data)	4	ABC, EC3, SMAN, SMWN
Skin sensitisation ECETOC	European Center of Ecotoxicology and Toxicology (ECETOC), Belgium	39	42	2	Respiratory sensitisation, Skin sensitisation
Dendritic cells COLIPA	European Cosmetics Association (COLIPA)	119	244	2	CD54, CD86
Keratinocyte gene expression Givaudan	Givaudan International AG, Switzerland	100	298	3	EC1.5, EC2, EC3
Chemical Reactivity COLIPA	European Cosmetics Association (COLIPA)	113	285 (106- % depletion of Cystine; 105- % depletion of Lysin; 74-Adduct Formation)	3	% depletion of Cystine, % depletion of Lysin, Adduct

					Formation
GSH Experimental RC50	Safety & Environmental Assurance Centre, Unilever; International QSAR Foundation; University of Tennessee, Knoxville, USA	424	1022	1	GSH RC50
Skin irritation	National Institute for Public Health and the Environment (RIVM), Netherlands; European Center for the validation of Alternative methods (ECVAM); European Center of Ecotoxicology and Toxicology (ECETOC),Belgium; School of Pharmacy and Chemistry, Liverpool John Moores University, UK	354	558	1	Primary Irritatation Index
Eye Irritation ECETOC	European Center of Ecotoxicology and Toxicology (ECETOC), Belgium	128	146	1	MMAS

Table 2. Datasets available in the OECD (Q)SAR Toolbox version 3.0.

Skin sensitization database.

Skin sensitisation database[4-8] includes two databases:

1. OASIS Skin Sensitisation includes 876 chemicals tested by Local Lymph Node Assay (LLNA), Guinea Pig Maximization Test (GPMT) or chemicals from the BfR list[4]. For 102 chemicals more than one skin sensitization data is stored in the database.

Based on the observed skin sensitization effect, the chemicals are classified to three classes:

- Class 2 - strong sensitizers. These are chemicals with EC3 < 10% in the LLNA test, or showing positive response in more than 30% of tested animals in the GPMT or classified as significant contact allergens by BfR (Category A).

- Class 1 - weak sensitizers. These are chemicals with EC3 within the range of 10 to 50% in the LLNA test, or showing a positive response in 1 to 30% of tested animals in the GPMT or possessing a solid-based indication for contact allergenic effects according to BfR (Category B).

- Class -1 - non sensitizers. These are chemicals without positive effects in the LLNA and GPMT or with insignificant/questionable contact allergenic effects according to BfR (Category C).

2. LJMU[9] database with skin sensitization data contains 212 chemicals tested by more than one test method. The chemicals are classified as strongly sensitizing (4 chemicals); moderately sensitizing (47 chemical)s; non sensitizing (155 chemicals) and ambiguous (6 chemicals).

Fig.1 shows the OECD (Q)SAR Toolbox tree, as appear to users when data from the Skin Sensitization database are gathered.

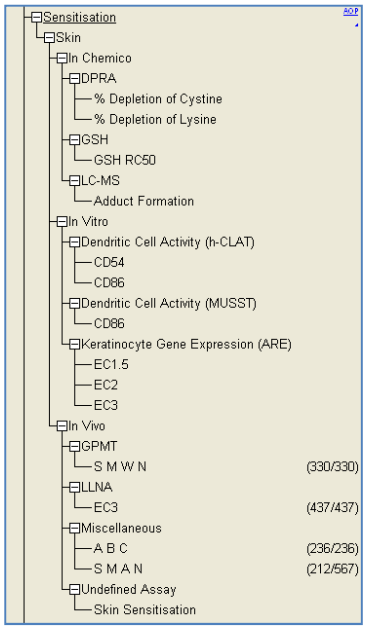

Figure 1. OECD (Q)SAR Toolbox tree: Skin Sensitization database.

Skin sensitization ECETOC database.

Skin sensitization ECETOC[10] database includes experimental results on skin and respirator sensitisation. Skin and Respiratory Chemicals Data Bank Report [11] contains a list of skin and respiratory sensitisers which may be used for the validation of *in vivo* or *in vitro* toxicological tests. The full list includes chemicals known as human and/or animal sensitisers, supported by published literature or other data from different available sources.

Figure 2 shows the OECD (Q)SAR Toolbox tree, as appear to users when data from the Skin Sensitization ECETOC database are gathered.

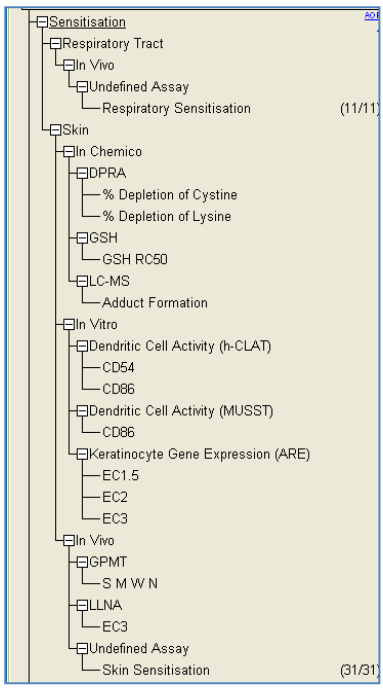

Figure 2. OECD (Q)SAR Toolbox tree: Skin Sensitization ECETOC database.

Dendritic cells COLIPA [12] database.

As noted in the AOP during allergen contact with the skin, immature epidermal dendritic cells, known as Langerhans cells, and dermal dendritic cells serve as antigen-presenting cells [13]. In this role, they recognize and internalize the hapten-protein complex formed during covalent binding. Subsequently, the dendritic cell loses its ability to seize new hapten-protein complexes and gains the potential to display the allergen- major histocompatibility complex (MHC) to naive T-cells; this process is often referred to as dendritic cell maturation. Additionally, during dendritic cell maturation MHC, co-stimulatory and intercellular adhesion molecules (e.g. CD86, and CD54) are up-regulated [14].

Alterations in intercellular adhesion molecules, cytokines and chemokines are part of the immunology response associated with skin sensitisation which can serve as biomarkers for skin sensitisation. *In vitro* expressions of these markers have been measured in endothelial cell-, keratinocyte- and especially dendritic cell-based cell lines.

Since dendritic cell maturation upon exposure to a sensitizing agent is accompanied by changes in surface marker expression, these surface markers are candidates as primary biomarkers of dendritic cell activation for the development of cell-based in vitro assays. Dendritic cell-like cell lines including the human monocytic leukaemia cell line (THP-1) and the human histiocytic lymphoma cell line (U937) have been used in biomarker studies aiming to distinguish known sensitizers from non-sensitizers.

While a variety of surface markers have been described to be up-regulated upon dendritic cell maturation, a review of the literature reveals that CD86 and CD54 expression are the most widely studied intercellular adhesion and co-stimulator molecules to date. The human cell line activation test (h-CLAT) [13, 15] reported flow cytometry results (i.e. positive or negative) for CD86 and CD54 expression in THP-1 cells treated with test chemicals. Briefly, cytotoxicity was determined using propidium iodide with flow cytometry analysis and five doses ones that produce 95, 85, 75, 65, and 50% cell viability were tested. If a material did not exhibit cytotoxicity the highest technical dose was assessed. Doses were subsequently tested in serial 1.3 dilution regime. A positive result was recorded if the relative fluorescence intensity of 1.5 times control for CD86 and 2. 0 times control for CD54 is observed.

The myeloid U937 skin sensitisation test (MUSST), is based as well on the measurement of CD86 by flow cytometry [16, 17]. The endpoint is defined by meeting a threshold of cell viability of 70% and positivity for CD86 with a EC150 (1.5 times) vehicle control in µg/ml.

Figure 3 shows the OECD (Q)SAR Toolbox tree, as appear to users when data from the Dendritic Cells COLIPA database are gathered.

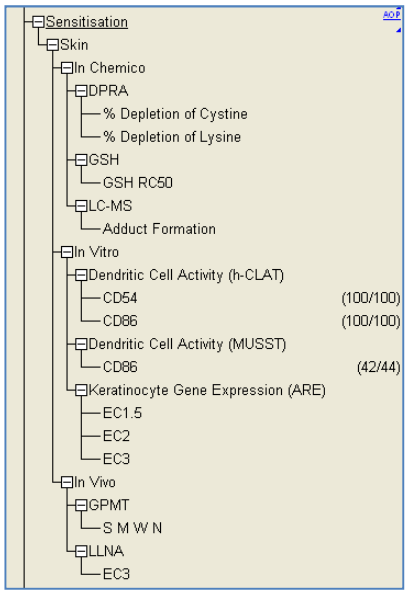

Figure 3. OECD (Q)SAR Toolbox tree: Dendritic Cells COLIPA database.

Keratinocyte gene expression Givaudan [18] database.

Electophilic haptens can react with cell surface proteins and activate response pathways in keratinocytes. Uptake of the hapten by keratinocytes activates multiple events, including the release of pro-inflammatory cytokines and the induction of cyto-protective cellular pathways.

Keratinocyte exposure to sensitizers can lead to the induction of antioxidant/electrophile response element ARE/EpRE-dependent pathways [19]. Briefly, reactive chemicals bind to Keap1 (Kelch-like ECH-associates protein 1) that normally inhibit the nuclear erythroid 2-related factor 2 (Nrf2). Released Nrf2 interacts with other nuclear proteins and binds to and activates ARE/EpRE-dependent pathways, including the cytoprotective genes NADPH-quinone oxidoreductase 1 (NQ01) and glutathione S-transferase (GSHST).

While several variants of the luciferase-based ARE assay have been developed, the *in vitro* reporter assay based on activation via the ARE/EpRE response element in keratinocytes is the basis for this profiler. Briefly, this so called KeratinoSens system of Natsch's group uses a luciferase reporter gene under control of a single copy of the ARE element of the human AKR1C2 gene stably inserted into immortalized human keratinocytes (h-CLAT). The experimental design is robust with chemicals routinely tested at twelve concentrations in triplicate from 1 to 2000 millimolar for 48-hours before evaluating for significant induction of gene activity [16]. Based on data derived from the assay three endpoints EC1.5, EC2 and EC3 (the concentrations eliciting a 1.5-, 2- and 3-fold increase in luciferase induction) are reported. If no gene induction is observed EC1.5, EC2, and /or EC3 are >2000.

Figure4 shows the OECD (Q)SAR Toolbox tree, as appear to users when data from the Keratinocyte Gene Expression Givaudan database are gathered.

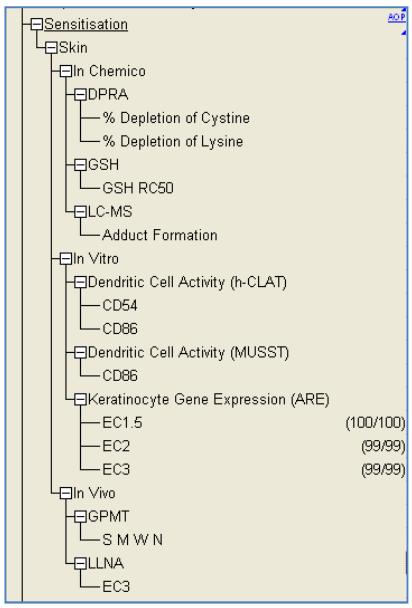

Figure 4. OECD (Q)SAR Toolbox tree: Keratinocyte Gene Expression database.

Chemical Reactivity COLIPA database.

Measurements of chemical reactivity largely fall into one of two types: depletion of a model nucleophile or monitoring of adduct formation. The advantage of the depletion approach is that it provides a uniform measurement parameter of tests based on depletion of a single nucleophile. For any given protocol, the same parameter is measured, and the same analytical methodology is applied, so the assay is easily standardized and often based on simple analysis which permits rapid and inexpensive comparisons to be made between arrays of test substances. However, with the depletion approach, no information is gained on the nature of the reaction taking place between the test material and the nucleophile and adduct formation is only assumed.

Given that the nature and location of the protein(s) relevant to skin sensitisation are currently unknown, it cannot be determined which particular model nucleophile, in which particular solvent, would provide the most realistic system for a particular test approach. However, a case can be made that the choice of the model nucleophile for determining reactivity is mainly dependent on how well the nucleophile performs in predicting sensitization potential. Test peptides can be designed to contain a single nucleophilic residue. This permits the determination of which nucleophile a given chemical has reacted with. Particular to DPRA (Depletion Peptide Reactivity Assay) is the use of cysteine and lysine residues [20]. Briefly, the system is based solely on the cysteine peptide at 1:10 and the lysine peptide at 1:50 with a selected test substance. The method measures depletion after a 24-hour reaction period with samples analysis by HPLC with UV detection and percent depletion being the reported endpoints. The cysteine and lysine peptides represent softer to harder model nucleophiles, which should help in detecting skin sensitisers which have different reaction mechanisms.

Particular to the Cor1-C420 Peptide Reactivity Assay is the use of Cor1-C420 peptide containing both the cysteine and lysine residues [21]. Briefly, in this assay 0.1 mM peptide and 10-fold excess of test substance are incubated for 24 h at 37°C and peptide depletion was measured using quantitative LC-MS. This modification of the original HPLC assay allows for simultaneous quantification of peptide oxidation (dimerisation) and characterization of adduct formation and thus generates a more detailed characterisation of the reactivity.

Figure 5 shows the OECD (Q)SAR Toolbox tree, as appear to users when data from the Chemical Reactivity COLIPA database are gathered.

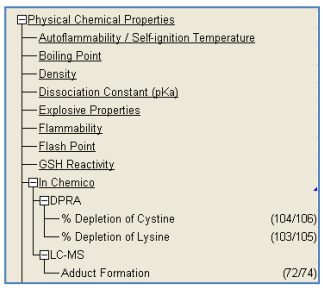

Figure 5. OECD (Q)SAR Toolbox tree: Chemical Reactivity COLIPA database.

GSH Experimental RC50 database.

Abiotic thiol reactivity expresses the in chemico RC50 value for electrophiles reacting via the Michael addition and Sn2 mechanisms. The RC50 value reflects the concentration of electrophile required so that half the available free thiol is bound to the target electrophile at the fixed time of 120 minutes. The RC50 is kinetic-based as it is inversely related the reactivity rate constant it can be thought of as the concentration of electrophile that if held constant gives a half-life of 120 minutes. Data are obtained by measuring target chemical covalent binding with the thiol group of glutathione (GSH) using a simple and rapid, spectrophotometric-based assay where the free thiol is quantified by measuring the absorption of the product at 412 nm after its reaction with 5,5'-dithio-bis(2-nitrobenzoic acid).

The empirical data for thiol reactivity presented in this data set show reasonable consistency in reactive potency within sub-structural groups (i.e., isoreactive groups). It allows for the quantification of relative thiol reactivity and identification of iso-reactive subcategories which can be relate to specific structural features. These data can be used to assist in read-across for skin sensitization and as a descriptor in trend analysis for acute toxic endpoints.

The database was tabulated and quality assured by the Biological-Activity Testing and Modeling Laboratory at The University of Tennessee, College of Veterinary Medicine, Knoxville, Tennessee USA. Quality assurance included evaluations of RC50 values including intra-chemical replicate comparisons (e.g., replicates of methyl acrylate), inter-group comparisons (e.g., between acrylates) and comparisons with kinetic rate constants. Key literature includes [22-29].

Figure 6 shows the OECD (Q)SAR Toolbox tree, as appear to users when data from the GSH Experimental RC50 database are gathered.

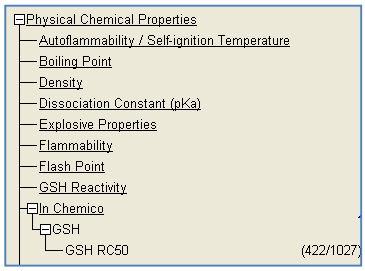

Figure 6. . OECD (Q)SAR Toolbox tree: GSH Experimental RC50 database.

Skin irritation database.

Skin irritation database includes two databases:

1. The RIVM [30] Skin Irritation database which contains Primary Skin Irritation Indices from skin irritation tests from the following sources:

- ECVAM Workshop 6: Corrosivity [31]
- ECETOC Technical Report No.66 Skin Irritation and Corrosion: Reference Chemicals Data Bank [32]
- Experimental results from [33]

2. Experimental results for Primary Skin Irritation Indices from LJMU [9].

Additional experimental results gathered from OECD SIDS Dossiers published between 1992 and 2009 were added in 2010 [7].

Figure 7 shows the OECD (Q)SAR Toolbox tree, as appear to users when data from the Skin Irritation database are gathered.

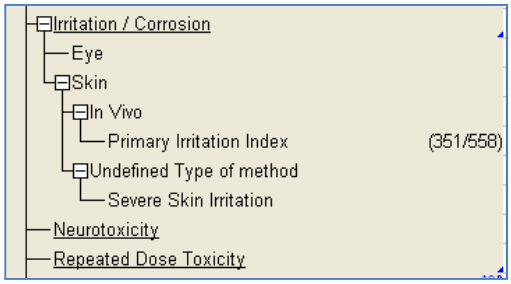

Figure 7. OECD (Q)SAR Toolbox tree: Skin Irritation database.

Eye Irritation ECETOC database.

This database contains experimental results on rabbit eye irritation from ECETOC report Eye Irritation Reference Chemicals Data Bank [34].

Figure 8 shows the OECD (Q)SAR Toolbox tree, as appear to users when data from the Eye Irritation database are gathered.

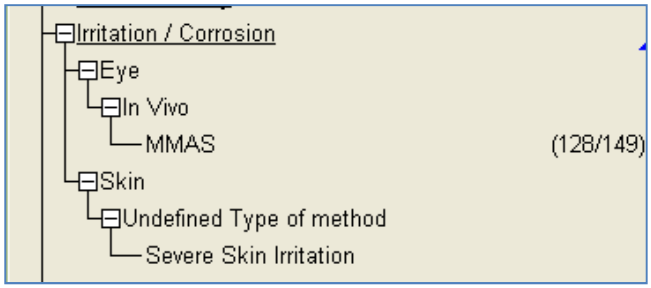

Figure 8. OECD (Q)SAR Toolbox tree: Eye Irritation database.

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1.2.2 Review of endpoints including resources and assays not present in the OECD (Q)SAR Toolbox

Skin sensitisation.

We did not identify any new databases for skin sensitisation endpoint (e.g. generated with novel in vitro assays described in this section) in addition to data already available in the OECD Toolbox v. 3.0.

Below is the detailed review of the endpoint and tools for risk assessment including methods that are currently under development or evaluation.

Skin sensitisation is a defined toxicological endpoint of regulatory significance. Current approaches to concretely assess skin sensitization potential, are carried out through in vivo testing. OECD Test Guidelines 429 and 406 describe the LLNA [35] and GPMT/Buehler[36] tests (OECD, 1992, 2002).

To meet the challenge of the REACH and the Cosmetics Regulation, there is the need of non-animal test methods able to generate information that could be used for skin sensitiser potency predictions.

Several non-animal methods are being developed for hazard identification to support hazard classification and labelling; however, data from these test methods alone will not be sufficient for risk assessment decision-making.

Skin sensitisation is associated with chemicals that have the intrinsic ability to cause skin allergy, termed allergic contact dermatitis (ACD) in humans. This adverse effect results from a reaction of the adaptive immune system and thus involves two phases, the induction of sensitisation which is followed upon further contact with the sensitising chemical by the elicitation of allergy symptoms. The symptoms of ACD usually develop in the area where the allergen actually touches the skin and include: red rash (the usual reaction) and blisters, itching and burning skin. Detailed reviews of the mechanistic aspects of skin sensitisation/allergic contact dermatitis can be found in literature [14, 37]. The main steps are shown in Figure 9 and involve

1 Skin bioavailability—the extent to which the compound reaches the site for haptenation.

2 Haptenation—the covalent binding of the chemical sensitiser to skin protein.

3 Epidermal inflammation—the release of pro-inflammatory signals by epidermal keratinocytes.

4 Dendritic cell (DC) activation—the activation and maturation of skin-associated DCs (i.e. Langerhans cells) in response to the combined effects of steps 1 and 2, including maturational changes to the DCs.

5 DC migration—the movement of hapten-peptide complex bearing dendritic cells from skin to the draining lymph node.

6 T-cell proliferation—the clonal expansion of T cells specific for the hapten-peptide complex.

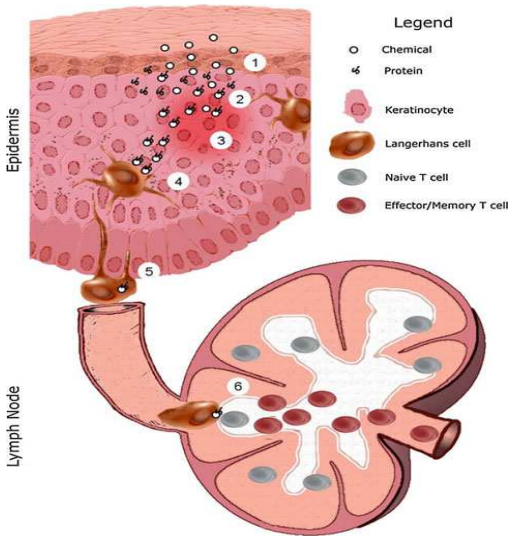


Figure 9. Main steps in the mechanism of skin sensitisation induction.

The numbers on the Figure 9 correspond to the steps described in the text. (1) Skin bioavailability, (2) haptenation, (3) epidermal inflammation, (4) DC activation, (5) DC migration, (6) T-cell proliferation. This figure contains elements of an image in the public domain from the National Cancer Institute [37].

Inventory of animal test methods currently available.

To date, there are no non-animal replacements available to definitively identify skin sensitization hazard or evaluate risk . In vivo methods are still used for the regulatory assessment of sensitization potential. The guinea pig maximization test (GPMT) and the Buehler test were for many years the tests of choice.

The GPMT is an adjuvant test in which the acquisition of sensitization is potentiated by the use of Freund's complete adjuvant (FCA) for intravenous exposure, combined with intradermal exposure. Guinea pigs are successively exposed to the test substance by intradermal injection (with and without Freund's complete adjuvant as immune enhancer) and topical application by occlusion (induction exposure). Following a rest period of 10-14 days (induction period), the animals are exposed dermally to a challenge dose using 24-h occlusion . The extent and degree of skin reactions to this challenge exposure is then compared with control animals. A rechallenge treatment may be considered 1-2 weeks after the first challenge to clarify equivocal results

The Buehler test is a non-adjuvant method requiring topical application for the induction phase. Guinea pigs are repeatedly exposed to the test substance by topical application under occlusion (induction exposures). Following a rest period of 12 days (induction period), a dermal challenge treatment is performed under occlusive conditions. The skin reactions to the challenge exposure are compared with the reaction in control animals . A rechallenge treatment may be considered 1-2 weeks after to clarify equivocal results.

For classification purposes, a response of at least 30% of the animals is considered positive for the GPMT and at least 15% in the Buehler test [38]. Both the GPMT and the Buehler test have been demonstrated to detect chemicals with moderate to strong sensitization potential as well as those with relatively weak sensitization potential. Details of the methods are given in the respective guidelines guideline 406 (OECD, 1992) [36].

More recently, the murine local lymph node assay (LLNA) as described in OECD guideline 429 (OECD, 2002) [35] has emerged as the preferred choice method for measuring skin sensitization potential. In fact, under REACH, the LLNA is stated to be the first choice method for in vivo testing. In the LLNA, skin sensitization hazard is defined as a function of the ability of the test chemical to provoke immune activation (lymphocyte proliferation) in lymph nodes draining the site of topical application [39-41]. The test substance is applied to the dorsum of the ears of mice for 3 consecutive days. On day 5, tritiated thymidine is injected intravenously as

a radioactive label for the measurement of cell proliferation. Five hours later, the auricular lymph nodes are excised and the incorporated radioactivity counted.

A substance is classified as a sensitizer if it induces a threefold stimulation index (SI) or greater at one or more test concentrations [42, 43]. The LLNA is also able to provide a reliable measure of relative skin sensitizing potency. Potency is measured by derivation of an estimated concentration of substance required to induce a threefold SI value (EC3) as compared with concurrent vehicle controls [43].

Non-animal tools for risk assessment

To determine whether a chemical has the potential to induce skin sensitisation, non-animal test methods are being developed which reflect the key mechanisms involved in skin sensitisation. It has been proposed that no single approach could predict sensitiser potency, and that the integration of multiple forms of non-animal data/ information would be necessary [44, 45].

There are ongoing research projects to develop generic strategies for integrating the results obtained from these different categories. The development of integrated testing strategies (ITS) for human health endpoints, including skin sensitisation, is one focus of the OSIRIS project [46] included under the Sixth Framework Programme funded by the European Commission. The Project arrived to conclusion in 2011. In addition, an important objective of the OSIRIS project was to develop a generic strategy for ITS including quantitative estimates of certainty. A review of ITS for skin sensitisation is provided in the JRC Scientific and Technical report [47], prepared as a contribution to the OSIRIS project.

Several *In silico* expert systems for predicting skin sensitisation are freely available.

The OECD (Q)SAR Application Toolbox [2] allows users to apply structure-activity methods to group chemicals into categories and then to fill data gaps by read¬across, trend analysis or external (Q)SARs. The key step in using the Toolbox is the formation of a chemical category , which is a group of chemicals whose physicochemical and human health and/or environmental toxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity. Filling the data gaps with the Toolbox is only possible when there is measured data for one or more chemicals within the category. Experimental results for skin sensitisation which are searchable by structure within the Toolbox are described in section 2.1.

The Toxtree software [48] allows identification of mechanisms of toxic action for skin sensitisation using a structure alerts approach [49].

Statistically based models for predicting skin sensitisation have been developed within the EU-funded CAESAR project [50] implemented into software and made available for online use.

The ability to make predictions on the skin sensitisation potential based on chemical reactivity has been recently evaluated under the UK Defra LINK project [51] (funded by the United Kingdom Department for Environment, Food and Rural Affairs).

Several expert systems which claim to predict skin sensitisation are commercially available.

DEREK expert knowledge base system [52] has an extensive rule base able to identify skin sensitisers. The rule base within Derek for Windows is mechanistically based taking the premise that haptenation is the key event that leads to skin sensitisation. Such systems are of great benefit in supporting other predictions (for example, predictions made by read across or statistical QSAR.

TIMES [53, 54] MEtabolism Simulator platform used for predicting skin sensitisation is a hybrid expert system that was developed at Bourgas University using funding and data from a consortium industry and regulators. TIMES-SS encodes structure- toxicity and structure-skin metabolism relationships through a number of transformations, some of which are underpinned by mechanistic three-dimensional quantitative structure-activity relationships.

The International QSAR Foundation [55] has created Effectopedia [56], a web-based tool to assist in the acquisition of biological response mechanisms and the associated biological knowledge bases associated with these mechanisms. They describe the most likely toxicity pathway. Skin sensitisations initiated by covalent binding to proteins is an example of relatively well-recognised endpoints, for which the AOPs are accurately developed. The AOP could provide a transparent, chemical and biological mechanistically-based framework for developing or refining chemical categories, as well as proposing and prioritising targeted in vitro and in vivo testing. By understanding the likelihood of effects at the chemical level and/or lower levels of biological organisation from structure-activity relationships (SARs), and *in chemico* and *in vitro* assays, one could efficiently determine if additional tests at higher levels of biological organisation (e.g. in vivo assays) are required [57].

Characterisation of skin sensitisation potential is a key endpoint for the safety assessment of cosmetic ingredients especially when significant dermal exposure to an ingredient is expected. At present the mouse local lymph node assay (LLNA) remains the 'gold standard' test method for this purpose however non-animal test methods are under development that aim to replace the need for new animal test data [58]. COLIPA (the European Cosmetics Association) funds an extensive programme of skin sensitisation research in order to propose guiding principles for the application and further development of non-animal safety assessment strategies [59].

The non-animal test methods currently under development or evaluation within the COLIPA [58]research programme or by other organisations are shown in Table 2.

These tests aim at replace the need for new animal test data through predicting how cosmetic ingredients will affect the various chemical or biological pathways identified as relevant to the induction of skin sensitisation in humans so far. At present, none of these in vitro test methods has been accepted by national or international regulatory authorities as a partial or full replacement for animal tests.

In vitro methods includes binding capacity towards proteins; responses of human cell types, i.e., primary keratinocytes (KC), dendritic cells (DC), and T cells or relevant immortalised cell lines in terms of bio-markers, cytokine secretion or gene expression (gene signature).

The direct peptide reactivity assay (DPRA) [20, 60] discriminates sensitisers (S) from non-sensitisers (NS) on the basis of their chemical reactivity towards two synthetic peptides (containing either a single cysteine or lysine as a reaction target) and by measuring their depletion using High Performance Liquid Chromatography. As an improvement to this assay an oxidation step has been included in the next generation of the DPRA assy using hydrogen peroxide in the presence or absence of horseradish peroxidase to oxidise the chemical under investigation to enable detection of pre- and pro-haptens [61]

The myeloid U937 skin sensitisation test (MUSST) uses flow cytometry to determine induction of the activation marker CD86 on the DC line U937 for S/NS discrimination [16].

More recently a concentration-dependent increase in intracellular IL-18 at non-cytototoxic concentrations of chemical was observed in the human keratinocyte cell line NCTC2455 following 24-h treatment [62]. Notably no changes in the baseline level of IL-18 were observed following treatment with respiratory allergens or

irritants indicating that cell-associated IL-18 may provide an in vitro tool for identification and discrimination of contact versus respiratory allergens and/or irritants. The approach developed in the framework of the EU FP6 Sens-it-iv project [63] is currently undergoing inter-laboratory evaluation to assess its transferability and reproducibility in discriminating between sensitising and non-sensitising chemicals, as well as its ability to estimate potency.

The human cell line activation test (h-CLAT) employs the DC line THP-1 and bases S/NS decision on CD86 plus CD54 induction [13, 15].

DPRA, MUSST and h-CLAT are part of an ECVAM Phase III pre-validation study [64] and some of the other tests, such as the tired approach (using keratinocytes and epidermal equivalents [62, 65]) and the KeratinoSens (using reporter gene activation in keratinocytes [66]), are short of entering pre-validation experiments.

Method	Developed by	Assay type
1. Toxicokinetic [67]	J. Kasting, Uni of Cincinnati, USA	In silico
2. DPRA [20, 60]	P & G	In chemico
3. MUSST [16]	L'Oreal	DC in vitro
4. h-CLAT [13, 15]	KAO&Shiseido	DC in vitro
5. NCTC IL-18 test [62]	Sens-it-iv	KC in vitro
6. Tiered approach [62, 65]	Sens-it-iv	KC and epidermal equivalent
7. AREc32[19]	CXR Biosciences	AREc32 cells in vitro reporter gene activity
8. MUTZ-3 Gene signature [68]	Sens-it-iv	DC in vitro gene expression
9. Peptide reactivity [69]	Unilever	In chemico
10. DPRA next generation [61]	P & G Univ. Strasbourg	In chemico
11. KeratinoSens [66]	Givaudan	KC in vitro, reporter gene activation
12. VITOSens™ [70]	CARDAM-VITO	DC in vitro Gene expression
13. PBMDC [71]	Beiersdorf	moDC in vitro
14. SenCeeTox™[72]	CeeTox	GSH depletion & KC in vitro gene expression
15. Multi parameter biosensor [73, 74]	Univ. of Toledo, Ohio, USA	DC in vitro, reporter gene activation
16. DC migration [75]	Imperial College London	DC in vitro, reporter gene activation
17. T cell proliferation [76]	Univ. Freiburg	PBMC in vitro
18. T cell proliferation [77-79]	Univ. Lyon	PBMC in vitro

Table 3. Alternative assays available within COLIPA and other organisations.

Skin irritation/corrosion.

We did not identify any new databases for skin irritation/corrosion endpoint (e.g. generated with novel in vitro assays described in this section) in addition to data already available in the OECD Toolbox v. 3.0.

Below is the detailed review of the endpoint and tools for risk assessment including methods that are currently under development or evaluation.

Skin irritation and skin corrosion refer to localized toxic effects resulting from a topical exposure of the skin to a substance.

Dermal Irritation is the production of reversible damage of the skin following the application of a test substance for up to 4 hours.

Dermal Corrosion is the production of irreversible damage of the skin; namely, visible necrosis through the epidermis and into the epidermis, following the application of a test substance for up to 4 hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions.

The review article by Weiss, et al. [80]describes the structure of the skin and the mechanisms of skin irritation.The outermost layer of the skin is called the epidermis. The epidermis is made up of approximately four layers of epithelial cells called keratinocytes. The epidermal keratinocytes and junctions between these cells form the barrier of the skin, preventing substances from penetrating the skin, and water and electrolytes from leaking out of the body. Keratinocytes undergo a process of differentiation as they move from the bottom epidermal cell layer to the top [81]. Terminal differentiation of the keratinocytes results in cornified (dead) keratinocytes on the surface of the skin forming the stratum corneum, another protective barrier for the skin. Langerhans cells, found in the epidermis, detect foreign antigens such as the molecules on the surface of invading microbes and travel to the lymph nodes where they present these antigens to cells of the immune system. Melanocyte cells, also in the epidermis, produce the melanin pigment that colors the skin.The innermost layer of the skin, called the subcutaneous layer, is relatively thick and primarily composed of fat cells. It is a source of insulation and physical protection for the body as well as a source of energy for the cells.

Animal Tests

Regulatory classification of skin irritation has historically been based on rabbit data. A test substance is applied to the shaved bare skin (about 6 cm2) of healthy young adult albino rabbits and the area is covered with gauze (OECD Test Guideline (TG) 404; Acute Dermal Irritation/Corrosion) [82]. The substance is removed after four hours and the rabbit's skin is observed at specific times for irritant responses for as many as 14 days. One animal is usually tested first. The grading of the skin responses by technicians is subjective, and is one source of the variability observed. Substances that are caustic (alkali) or of extreme pH are typically classified as corrosive without animal testing.

Regulatory Requirements and Test Guidelines

The Organisation for Economic Co-operation and Development (OECD) adopted a tiered approach for dermal testing in 2001, and this approach is described in the revised TG 404 (April 24, 2002) [82]. TG 404 recommends the use of validated in vitro or ex vivo methods when appropriate. The weight-of-evidence approach includes analysis of all existing human and animal data, pH extremes, and in vitro data.

Human data and experience, screening assays and pH extremes (to identify obvious corrosive materials), and data from structurally related materials are sometimes sufficient for classifying the skin irritation/corrosion potential of a substance. If this information is not sufficient, validated in vitro methods can then be used.

Non-animal Alternative Methods

A variety of cell-based methods have been developed and used for the assessment and/or ranking of skin irritants [81]. Cell models include monolayer cultures of human and animal skin cells (keratinocytes), multilayered (3-dimensional (3D)) cultures of skin cells that provide a barrier function like the surface of the skin, and co-culture models where two or more of the types of cells found in the skin are represented. A recent review article describes the features and evolution of the 3D skin models composed of human keratinocytes cultured at the air-liquid interface to induce stratification and development of a barrier function [83]]. Research is also underway into the use of stem cells to create or regenerate in vitro skin cell models.

Commercial in vitro skin models are now available for conducting reproducible in vitro assessments, and several of the models have been endorsed as scientifically valid for certain testing applications.

The 3D skin models, or reconstructed human epidermis (RHE) models, consist of human cells grown on a membrane at the air-liquid interface. This method of culture induces the cells to grow in multilayers and to form junctions between the cells so that the cultures are similar to mini pieces of human skin in the wells of a petri plate. Skin models that are commercially available include the following: EpiDerm Skin Irritation Test (SIT) (MatTek Corporation, Ashland, MA, US), EpiSkin SIT (SkinEthic, Nice, France), SkinEthic RHE (SkinEthic, Nice, France), LabCyte EPI-MODEL (J-TEC, Japan), EST-1000 RHE (Advanced CellSystems, Germany), and Vitrolife-Skin RHE (GUNZE Medical Division, Japan).

The endpoints typically evaluated for skin irritation testing are cytotoxicity (MTT assay), or cytotoxicity (MTT assay) plus IL-1a (cytokine release). The endpoint evaluated for skin corrosion is cytotoxicity (MTT assay).

Ex vivo models, or skin explants, consist of pieces of skin from humans or animals for in vitro testing applications. These have been used in screening for skin irritants but are more useful for testing skin corrosion or dermal absorption (skin penetration). Examples of this type of model used for skin irritation testing are the mouse skin integrity function test (SIFT), human skin from surgery, and the pig ear test [84]. The rat skin transcutaneous electrical resistance (TER) method has been validated for skin corrosion testing.

Human volunteer skin testing (patch testing) can be ethically conducted on products and ingredients where the hazard of the substance is already substantially understood [85, 86]. Generally only mild substances would be tested for irritation in this way, and no materials thought to be corrosive could be assessed on human volunteers. A database of human skin irritation data from 4-hour patch tests for 65 substances has been published, which could be useful in the validation of human cell-based methods [85].

(Quantitative) structure-activity relationship ((Q)SAR) models have been developed [84, 86] for skin irritation studies but are not commonly used.

In 1999, an ICCVAM review of Corrositex recommended its use as a stand-alone assay for evaluating acids, bases, and acid derivatives for the US Department of Transportation, and otherwise, as part of a tiered testing strategy. A reciprocal statement of validity was issued by ESAC in 2000. That same year, Corrositex achieved formal acceptance by US regulators, and in 2006, the method achieved international acceptance as OECD TG 435[87].

EpiSkin, EpiDerm and the rat TER tests were validated by ECVAM and endorsed by the ESAC in 1998 as "scientifically validated for use as a replacement for the animal test, and … ready to be considered for regulatory acceptance." These tests underwent a subsequent validation review by ICCVAM, which in 2002 recommended their use as part of a tiered testing strategy. They achieved formal EU acceptance for regulatory purposes in 2000, followed by international acceptance as OECD TGs 430 [88] and 431 [89] in 2004. A Draft

Revised TG 431 is currently under consideration, which includes a technical update on performance standards and recommends substitution of two reference chemicals.

Variants of the EpiSkin and EpiDerm corrosivity tests were evaluated for their ability to predict skin irritation [90]). On the basis of this validation study, concluded that the EpiSkin Skin Irritation Test (SIT) "showed evidence of being a reliable and relevant stand-alone test for predicting rabbit skin irritation...and for being used as a replacement...for the Draize Skin Irritation Test...for the purposes of distinguishing between R38 skin irritating and no-label (non-skin irritating) test substances." With the protocol used in this study, the EpiDerm SIT test was endorsed as a component of a tiered testing strategy, in which negative results required further confirmation.

Methods*	Test Purpose	Validation Authority
EpiSkin SIT	Skin irritation	ECVAM
EpiDerm SIT	Skin irritation	ECVAM
Modified EpiDerm SIT	Skin irritation	ECVAM
SkinEthic RHE	Skin irritation	ECVAM
LabCyte EPI-MODEL 24	Skin irritation	JaCVAM; OECD
EpiDerm - in vitro human skin	Skin corrosion	ICCVAM[91]; ECVAM [92](INVITTOX protocol)
EpiSkin - in vitro human skin	Skin corrosion	ICCVAM; ECVAM (INVITTOX protocol)
Rat Skin Transcutaneous Electrical Resistance (TER)	Skin corrosion	ICCVAM; ECVAM (INVITTOX protocol)
Corrositex - noncellular membrane	Skin corrosion	ICCVAM; ECVAM (INVITTOX protocol)
SkinEthic - in vitro human skin	Skin corrosion	ECVAM
Vitro-Life Skin	Skin corrosion	JaCVAM
EST-1000	Skin corrosion	ESAC

Table 4. Non-animal test methods for skin irritation/corrosion.

Eye irritation/corrosion.

We did not identify any new databases for eye irritation/corrosion endpoint (e.g. generated with novel in vitro assays described in this section) in addition to data already available in the OECD Toolbox v. 3.0.

Below is the detailed review of the endpoint and tools for risk assessment including methods that are currently under development or evaluation.

One component in the safety assessment of many types of products is the evaluation of their potential to cause eye injury. Eye irritation is defined as "the production of changes in the eye following the application of test substance to the anterior surface of the eye, which are fully reversible within 21 days of application". Eye corrosion (serious eye damage) is defined as "the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application" (UNECE, 2004) [93].

The anterior surface of the eye is covered by the cornea and the conjunctiva. The cornea is the most exposed area and, therefore, the most likely part of the eye to be involved in a chemical exposure to the eye. Chemical injury to the cornea can result in the loss of vision. Accordingly, corneal injury in the animal test for eye irritation/corrosion accounts for 73% of the total ocular toxicity score. For these reasons, the cornea has been the primary tissue modeled in vitro alternative models for accessing eye injury.

Many mild eye irritants act by disrupting or damaging only the surface cells of the corneal epithelium, and the corneal can repair this type of damage within a short time. The stronger the eye irritant, the deeper it penetrates into the next layer of the cornea, the stroma. Very damaging materials might penetrate deep enough to cause irreversible injury, including damage to the corneal endothelial cell layer, where tight junctions modulate the penetration of water and other substances from the cornea to the aqueous humor.

The conjunctiva covers the remaining surface of the eye and is also important in protecting the eye from environmental insults. Injury to the conjunctiva has been assigned about 18% of the in vivo eye injury score in the Draize test but has largely been ignored in in vitro assessments of chemical eye injury. Conjunctival injury may be irrelevant in moderate to severe eye injury, where the corneal effects are largely predictive of reversibility and outcome, but could be useful in assessing milder effects, especially for products used in and around the eye [94], for example, the conjunctiva can exhibit different mechanisms of injury than the cornea due to its different physiology. The conjunctiva contains goblet cells, which secrete the mucin layer that protects the surface of the eye, as well as immune and vascular components important in the eye irritation response.

The iris is the third ocular tissue assessed for response to an irritant in the in vivo eye test. It is generally agreed that an in vitro iris assessment for most substances is not necessary, as iris responses occur only upon significant disruption to the ocular surface barrier of the cornea [95] Therefore, the degree of corneal injury should be predictive of potential iris effects.

Animal Tests

Developed in 1944, the Draize rabbit eye irritation test remains the standard method for evaluating the ocular irritation/corrosion potential of a substance for regulatory purposes [87]In this test, a material is instilled into one eye of albino rabbits (the other eye serving as the negative control), and the response of the animals is monitored using a standardized scoring system for injury to the cornea, conjunctiva, and iris. Ocular responses are scored at 1, 24, 48, and 72 hours. The animals are observed until the full magnitude and reversibility of the ocular injury can be evaluated—for up to 21 days. Reversibility of the ocular injury is an important component in the classification of a substance as an eye irritant versus an eye corrosive. Different modifications of the test require different numbers of animals, although no more than three animals is the current standard.

Regulatory authorities in most countries require ocular safety assessments and commonly have some version of the Draize rabbit eye test as part of their testing guidelines. Draize rabbit eye data has proved to be highly variable, generally over predictive of human eye injury, and sometimes incorrect due to species differences in the ocular response to specific substances. However, there has been renewed interest in a variant of the

traditional Draize test, the low-volume eye test (LVET). In this test, one-tenth the dosing volume of the traditional test is placed directly on the cornea, as opposed to the conjunctival sac. In addition to giving responses closer to those observed in humans, it has the potential utility of providing mechanistic data [96].

Non-animal Alternative Methods

A number of non-animal test methods have been developed in the search for a replacement for the Draize rabbit eye test. Current non-animal methods considered valid for regulatory testing purposes (for limited applications) are listed in Table 3.

Method	Test Purpose
Bovine Corneal Opacity and Permeability (BCOP) assay [97, 98]	Eye corrosion/severe irritation
Isolated Chicken Eye (ICE) assay [99]	Eye corrosion/severe irritation
Isolated Rabbit Eye (IRE) assay [100]	Eye corrosion
Hen's Egg Test—Chorio-Allantoic Membrane assay (HET-CAM) [101]	Eye corrosion

Table 5. *In vitro* ocular test methods considered valid for limited regulatory testing applications.

1.2.3 Review of toxicological standard schemas

The most important standard that will be preserved during the ontology implementation is the OECD Harmonized Templates (OECD HTs) [102].

The original terminology of OHTs and databases vocabulary should be maintained in order to fulfill the requirements of the contract and to facilitate the implementation in the QSAR Toolbox.

The OECD HTs correspond to the IUCLID5 [103]XML schemas, which are meant to be used by industry when submitting documentation on their chemicals to EU regulatory authorities. For each endpoint, the OECD HTs describe a series of fields defining the information submission requirements of a experiment. Since they are generic enough to be able to include data on endpoints with different characteristics, in principle the OECD HTs provide a substantial basis for building ontology. However, when relative picklists [104] are not available; HTS leave much space for free text entering and need to be extended during their conversion to the ontology.

For the endpoints from SC2 the following HTs are available:

OECD Template #64: Skin irritation / corrosion

OECD Template #65: Eye irritation

OECD Template #66-1: Skin sensitisation

OECD Template #66-2: Respiratory sensitisation

In addition the proposal for a template for adverse outcome pathways [105] should be also taken in account.

The survey of ontology-related resources has pointed to another important toxicological standard, that is publicly available and close to the needs of a Toxicology Ontology: the ToxML schema (Lhasa Limited) [106]. The ToxML project proposes the definition of a common standard for the storage and exchange of toxicology data as a structured XML file format (ToxML file). The ToxML file is intended for transfer of experimental data (Compound and study data) between software applications.

ToxML schema[107] includes three toxicity study which are closely related to the Project:

LLNA Studies (Figure 10);

Skin Penetration Studies

Irritation Studies

ToxML is an XML data exchange standard based on toxicity controlled vocabulary. The most recent ToxML release has a comprehensive, well-structured structure. However, it should be emphasized that ToxML is not an ontology, since it contains only elementary relations, has no restriction rules and lacks of systematic definitions.

Figure 10. Screenshot of the ToxML LLNA assay schema.

1.2.4 Review of related ontologies

During the Project implementation we will identify the common classes that can be re-used from the ontologies developed under the Specific Contract No 1 (Carcinogenicity, Repeated Dose Toxicity and Reproductive/Developmental Toxicity). These classes could include the common HTs parts, the species/strains,

organs description, whereas toxicity tests covered by the Specific Contract No 2 are completely different from those from the Specific Contract No 1.

The toxicological ontology elaborated previously within the OpenTox project [108] does not cover skin and respiratory sensitization, skin irritation/corrosion, eye irritation/corrosion endpoints.

The search of the related ontologies have been performed at the Bio-portal ontology depository [109]. No systematic description of the endpoints of interest has been found. Some non systematic use of relative terms has been identified in several ontologies (see an example on Figure 11.) in several ontologies, e.g. MedDRA ontology [110], Clinical Terms Version 3 (CTV3) ontology [111], Medical Subject Headings (MeSH) [112].

These terms may not be very useful for the ontology building, but could be use for the synonyms collection, etc.

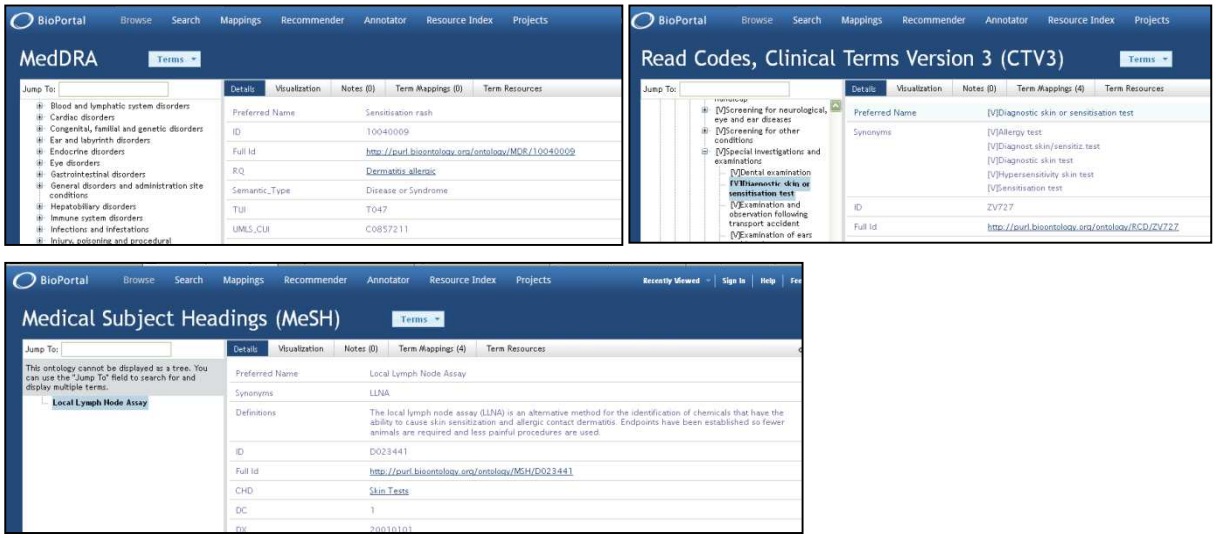


Figure 11. Screenshots from the Bioportal website.

1.2.5 Conclusions

Currently, no systematic ontology, covering the toxicological endpoints considering in the Specific Contract No 2 (skin and respiratory sensitization, skin irritation/corrosion, eye irritation/corrosion), is available.

Some common classes (regulatory tests description, organs) can be imported from ontologies developed under the Specific Contract No 1 (Carcinogenicity, Repeated Dose Toxicity and Reproductive/Developmental Toxicity).

However, significant part of work has to be done from scratch in order to cover all toxicity databases included in the OECD (Q)SAR Toolbox.

During the ontology development we will conserve the original OECD HTs and the HTs' picklists terminology. All resources described in the Section 2 will be used for HTs extension, synonyms definitions, etc., when applicable. All datasets currently present in the version 3 of OECD QSAR Toolbox will be completely covered from the ontological point of view and all terms will be mapped to the OECD HTs.

We did not obtain any information about the existence of new systematic databases, e.g. We did not obtain any information about the existence of new systematic databases, e.g. data generated with novel in vitro assays described in Section 2.2.

We will develop vocabulary/ontology for those new in vitro assays where information is available despite the absence of actual database which can be further consider in the long future once date become available. This will be like a separate ontology with specific annotation to enable distinction from the actual ontology of the Toolbox databases and will contain names of assays and other available information on the assay as such.

The ontology concept will be kept flexible and it will be possible to extend the ontology to cover new datasets when and if available in the next future.

1.3 Detailed work packages: Deliverables 1.2-1.4.

1.3.1 General considerations

The objective of each work package WP is to develop an ontology following the principles of the Open Biomedical Ontologies Foundry (OBOF) [3] and using the Web Ontology Language (OWL). A first requirement is obviously the compatibility of the Ontology with the existing terminology used in the OECD (Q)SAR Toolbox and the flexible ontology approach suitable for further possible extensions.

We propose to develop the ontologies in the following order:

WP1:Development of ontology for the first endpoint (skin irritation/corrosion)

WP2:Development of ontology for the second endpoint (eye irritation/corrosion);

WP3:Development of ontology for the third endpoint (skin and respiratory sensitization)

Each WP associated with one of three ontologies will be three months in duration and will include 5 tasks (deliverables):

1.3.2 Definition of classes and hierarchical relationships in the ontology structure

The contractor shall define classes and subclasses for the ontology and its hierarchical relationships, taking into account existing work as agreed in the inception phase. The resulting ontology structure should be flexible in order to be able to cover toxicity databases relative to the endpoints mentioned above that will be available in the (Q)SAR Toolbox version 3 (Table 1), as well as potential further extensions.

We will conserve as much as possible the structure of the OECD Harmonised Templates: [102]

OECD Template #64: Skin irritation / corrosion

OECD Template #65: Eye irritation

OECD Template #66-1: Skin sensitisation

OECD Template #66-2: Respiratory sensitisation

1.3.3 Compilation of terms related to the endpoint

Taking into account the results from the review of existing relevant work related to ontology, the contractor shall compile a list of related terms. It will include all terms relative to the endpoints - and related databases available in the version 3 of the OECD (Q)SAR Toolbox (Table 1), as well as in related OECD harmonised templates and picklists and other identified sources. These resources will include related existing ontologies freely available at the Bio-portal ontology depository [109] and, if applicable, ontologies developed under the Specific Contract No 1 (Carcinogenicity, Repeated Dose Toxicity and Reproductive/Developmental Toxicity)

We will take in account all currently available assays described in the section 2.2.

1.3.4 Definition of synonyms and homonyms

For each term, a maximum number of synonymous and homonymous terms shall be identified taking into account terms used in test guidelines, databases and other identified relevant sources. Synonyms and homonyms collection is very important for understanding how the same terms are defined in different datasets. This will further help to perform the mapping between datasets and ontology entries. The synonyms collection will be performed from all available resources will include related ontologies freely available at the Bio-portal ontology depository [109] and, if applicable, ontologies developed under the Specific Contract No 1 (Carcinogenicity, Repeated Dose Toxicity and Reproductive/Developmental Toxicity), existing standards, assays and databases, described in the sections 1-2.

1.3.5 Establishment of relationships, interactions and hierarchies between classes, objects and numerical properties for each term and rules when existing (internal rules and restriction rules).

The rules and restrictions, for classes, objects and numerical properties, within and across classes will be established where applicable. The exact mapping between the same terms coming from different databases and association of each attribute in a toxicological datasets with an entry in the ontology will be performed.

1.3.6 Reports

Intermediate ontology (OWL file in Protégé software) and report (i.e. word file) will be prepared

All deliverables for each WP should be approved by Ontology Coordination Group.

The final ontology report and final version of all three ontologies will be presented at the end of the Project

1.4 Detailed acceptance plan: Deliverables 1.5

All deliverables of each work packages are to be approved by the Ontology Coordination Group.

The contractor (ISS) will send the deliverables report in time required by the time line of the Project. Then the Coordination Group will identify any significant deficiencies precluding the acceptance of the deliverable.

During the face to face meeting and the regular teleconferences, the Contractor will update the Coordination group.

1.5 Detailed quality assurance plan: Deliverables 1.6

Detailed Quality Assurance Plan (QAP) will provide a clear view on how the work quality will be assured and will be maintained throughout the project. The main points of the QAP are described below.

The project will be managed via an Ontology Coordinating Group which will consist of one representative from the European Chemical Agency (ECHA) and one from the Organization for Economic Cooperation and Development (OECD).

The planning and other organizational aspects of the project, including a time line, for the entire project will be established during the kick-off phase with the OECD Project Manager and the ECHA Project Manager.

During the regular teleconferences, the Contractor will give an update to the Coordination group. The project status and the activities for the subsequent months and the technical issues regarding the ontology development will be discussed. The Contractor will present the project work also during the Ontology Coordination Group Meetings, and (Q)SAR Toolbox Management Group.

The contractor (ISS) will present all deliverables at milestones to the Coordination Group, to identify any significant deficiencies that would preclude the acceptance of the deliverable.

Since ontology is closely linked to the OECD QSAR Toolbox, the contractor will work closely with the OECD Toolbox contractors (LMC) to ensure that the three ontologies can be easily mapped to the Toolbox.

During the ontology development, external experts (e.g. experts from Obo Foundry, or The National Center for Biomedical Ontology as well as toxicology experts in the field of skin and eye irritation, skin and respiratory sensitisation) may be asked to review the quality of the ontological output.

During the inception phase, the Contractor will control the quality of all existing resources that could be re-used for the ontology development.

In last two months of the Project the final ontology will be sent to the external expert for reviewing.

1.6 Detailed time line for entire project: Deliverables 1.7

We propose the following list of milestones/tasks and timing:

Milestone/Task	**Starting Month**	**Ending Month**	**Deliverable**
1.Inception	**0**	**2**	
1.1	0	2	Review of existing work
1.2	0	2	Detailed work package l
1.3	0	2	Detailed work package 2
1.4	0	2	Detailed work package 3
1.5	0	2	Detailed acceptance plan
1.6	0	2	Detailed quality assurance plan
1.7	0	2	Detailed time line for entire project
2.WP1 Development of ontology for the first endpoint (skin irritation/corrosion)t	**3**	**6**	
2.1	3	4	Definition of c1asses and general hierarchical relationships in the

			ontology structure
2.2	3	5	Compilation of terms related to the endpoint
2.3	3	5	Definition of synonymous and homonymous
2.4	3	6	Establishment of relationships, interactions and hierarchies between classes, object and numeric properties for each term and rules
2.5	3	6	Intermediate ontology (i.e. OWL file) and report (i.e. word file)
3.WP2 Development of ontology for the second endpoint (eye irritation/corrosion)	**5**	**8**	
3.1	5	6	Definition of classes and general hierarchical relationships in the ontology structure
3.2	5	7	Compilation of terms related to the endpoint
3.3	5	7	Definition of synonymous and homonymous
3.4	5	8	Establishment of relationships, interactions and hierarchies between classes, object and numeric properties for each term and rules
3.5	5	8	Intermediate ontology (i.e. OWL file) and report (i.e. word file)
External Review of Irritation Ontology WP1&WP2			
4.WP3 Development of ontology for the third endpoint (skin and respiratory sensitisation)	**7**	**10**	
4.1	7	8	Definition of classes and general hierarchical relationships in the ontology structure
4.2	7	9	Compilation of terms related to the endpoint
4.3	7	9	Definition of synonymous and

			homonymous
4.4	7	10	Establishment of relationships, interactions and hierarchies between c1asses, object and numeric properties for each term and rules
4.5	7	10	Intermediate ontology (i.e. OWL file) and report (i.e. word file)
External Review of Sensitisation Ontology WP3			
4.6	**10**	**12**	**Final ontologies and final report**

Table 6. The list of milestones/tasks and timing of the Project.

2. WP1 Development of ontology for the Skin irritation/corrosion endpoint

2.1 Background

The goal of the WP1 is to develop ontologies suitable for standardizing and organizing the chemical toxicological databases in the OECD (Q)SAR Toolbox [2] for skin irritation/corrosion. The Open Biomedical Ontologies Foundry [3] principles, the Web Ontology Language (OWL) and the Protégé software are be used for the implementation.

During the Inception Phase of the Project (WP1) we have identified a number of related resources. A number of different tasks and subtasks have been identified as well.

The most important standard that should be preserved during the ontology implementation is the OECD Harmonized Templates (OECD HTs) [102].

The original terminology of OHTs and databases vocabulary should be maintained in order to fulfill the requirements of the contract and to facilitate the implementation in the QSAR Toolbox.

A first requirement of the ontology is obviously the compatibility of the Ontology with the existing terminology used in the OECD (Q)SAR Toolbox and the flexible ontology approach suitable for further possible extensions. Currently the OECD (Q)SAR Toolbox version 3.0 [2] contains only a skin irritation database containing 354 chemicals. The skin irritation database includes two datasets: the RIVM [30] Skin Irritation database which contains Primary Skin Irritation Indices from skin irritation tests and Experimental results for Primary Skin Irritation Indices from LJMU [9]. Additional experimental results gathered from OECD SIDS Dossiers published between 1992 and 2009 were added in 2010 [7].

As reported during the Inception Phase, we were not able to identify any new databases for the skin irritation/corrosion endpoint (e.g. generated with novel in vitro assays described in this section) in addition to data already available in the OECD Toolbox v. 3.0.

The Organisation for Economic Co-operation and Development (OECD) adopted a tiered approach for dermal testing in 2001, and this approach is described in the revised TG 404 (April 24, 2002) [82]. TG 404 recommends the use of validated in vitro or ex vivo methods when appropriate. These assays [113] are described in TG 430: in vitro skin corrosion: transcutaneous electrical resistance test, TG 431: In vitro skin corrosion: Human skin model test, TG 439: In vitro skin irritation: reconstructed human epidermis test methods and TG 435: In vitro Membrane Barrier Test method for skin corrosion [88, 89, 114, 115].

We will develop vocabulary/ontology for those new in vitro assays despite the absence of actual database which may be further considered in the future once date become available. This will be like a separate ontology class with specific annotation to enable distinction from the actual ontology of the Toolbox databases and will contain names of assays and other available information on the assay as such.

2.2 WP1 Skin irritation/corrosion: Deliverables 2.1 "Definition of classes and hierarchical relationships in the ontology structure". Detailed work description.

During the WP1 D2.1 implementation we have indentified and defined classes and hierarchical relationships for the skin irritation/corrosion ontology.

The present ontology has to respect regulatory needs, and was tailored on existing constraints, i.e.,: a) the OECD HTs; and b) the skin irritation database in the Toolbox. The goal of the project is to support and improve data integration and mapping in Toolbox, which is very domain-specific task, with most terms/concepts already having an official terminology (used e.g., in the OECD HTs).

To guarantee the compatibility of ontology with the OECD QSAR Toolbox, the ontology was separated into two main super-classes: the first one is dedicated to HT # 64 Skin irritation/corrosion, while the second covers the experimental datasets and assays terminology. Using this approach, it is possible to add smoothly any new skin irritation/corrosion dataset to the ontology.

The most important standard that has been preserved during the ontology implementation is the OECD Harmonized Templates (OECD HTs) [102]. We have conserved all documentation and the structure of the OECD Harmonised Template #64: Skin irritation / corrosion [102] and relative picklists [104] (Figure 12).

The conversion of the HT 64 into the OWL format was performed semi-automatically, with the Protégé Excel Import Plug-in. The ontology class based on OECD HT # 64: has five main sub-classes: Overall remarks, attachments, Applicant's summary and conclusion, Data source, Materials and Methods, Results and Discussion. The OECD field number and other information contained in the HTs documentation was maintained in order to facilitate the ontology maintenance in case of HTs updates.

When possible we have imported the SC1 Ontologies parts mostly for the overlapping administrative terms of the HT. The rest of work has been done from scratch.

The second part of the ontology "Ontology for experimental assays" covers

- the skin irritation database from the OECD QSAR Toolbox software v.3,

-new in vitro assays despite the absence of actual database, which can be further considered once date become available,

-OECD Test Guidelines for skin irritation/corrosion in vivo and in vitro.

The skin irritation database is included in the OECD (Q)SAR Toolbox v. 3.0. All terms of the databases have been translated in OWL format (Figure 13). This dataset will be further mapped to the OECD HT 64 ontology.

Based on a careful preliminary survey we did not obtain any information about the existence of new systematic databases, e.g. data generated with novel in vitro assays. Therefore it has been decided to develop vocabulary/ontology for new in vitro assays despite the absence of actual database. We have included the vocabulary for these assays in separate ontology class "In vitro test methods" (Figure 14). In such a way the ontology concept will be kept flexible and it will be possible to extend the ontology to cover new datasets when and if available in the next future.

The subclass "OECD Test Guidelines" covers the following Test Guidelines for skin irritation/corrosion (Figure 15):

1. OECD TG 430: in vitro skin corrosion: transcutaneous electrical resistance test
2. OECD TG 431: In vitro skin corrosion: Human skin model test
3. OECD TG 404: In vivo skin irritation/corrosion

4. OECD TG 439: In vitro skin irritation: reconstructed human epidermis test methods
5. OECD TG 435: In vitro Membrane Barrier Test method for skin corrosion

The general structure of the skin irritation/corrosion ontology is shown in Figure 16.

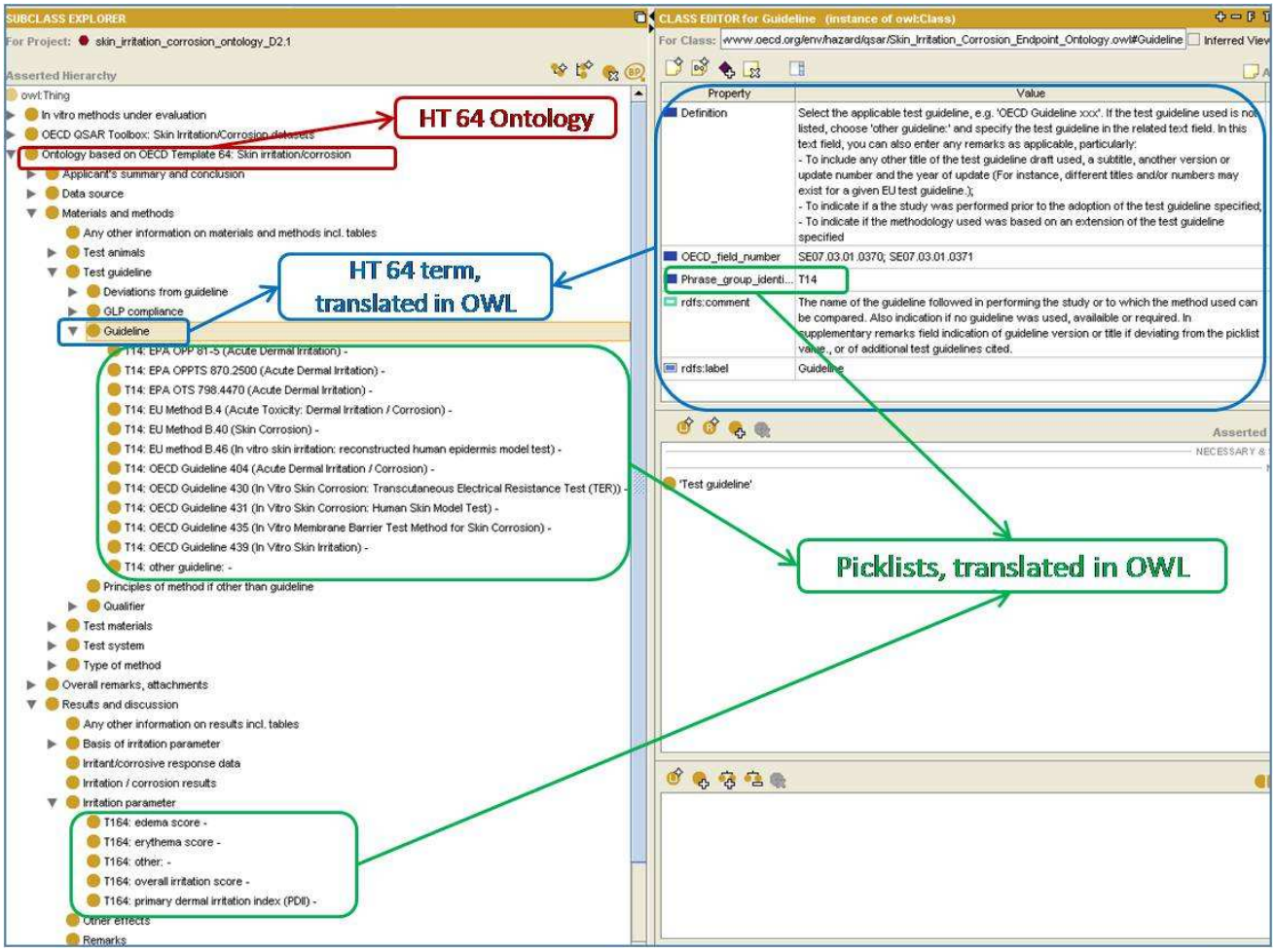


Figure 12. WP1.Protégé Screenshot: the HT's fragment in OWL format.

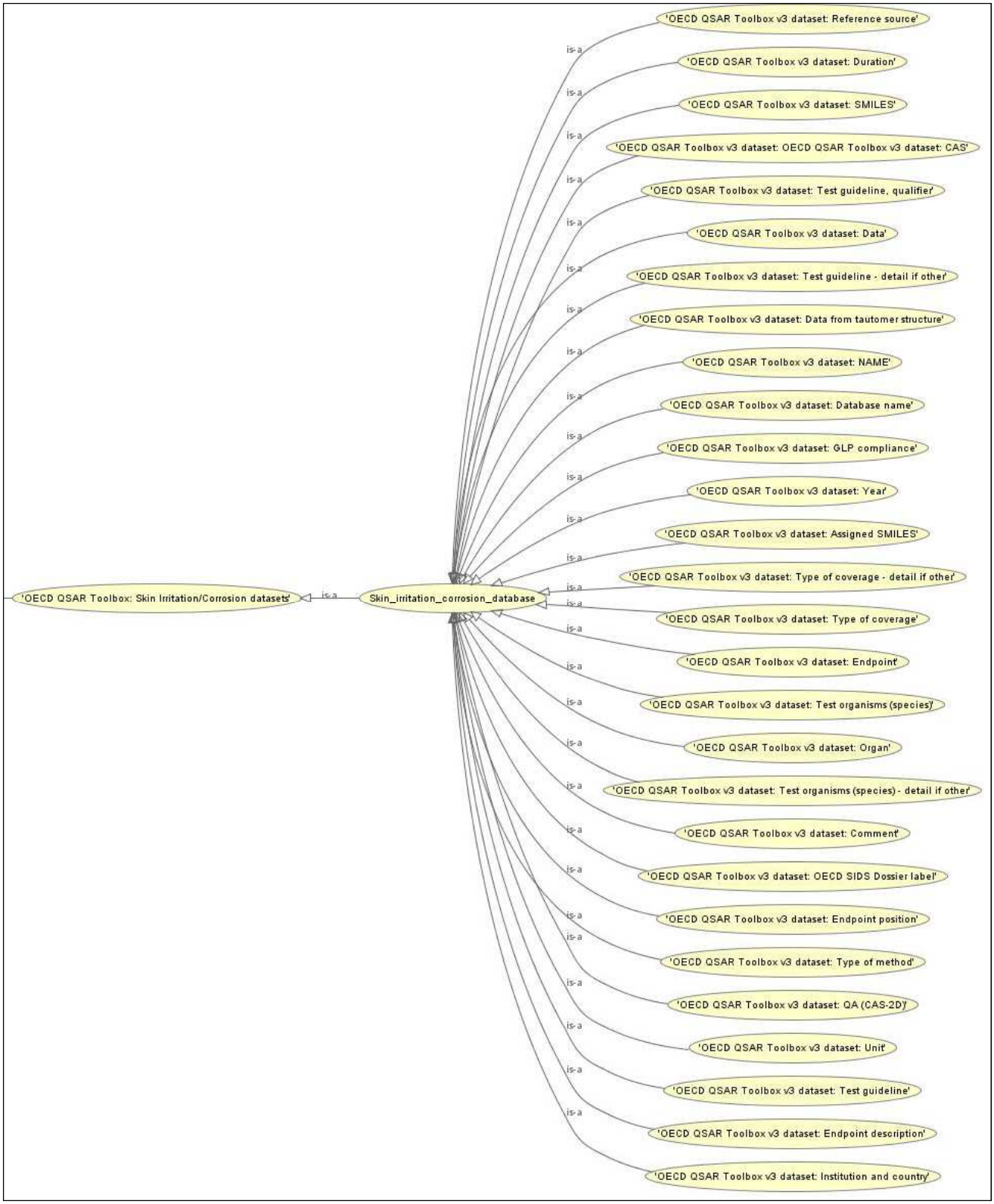


Figure 13. WP1.Main sub-classes describing the skin irritation database.

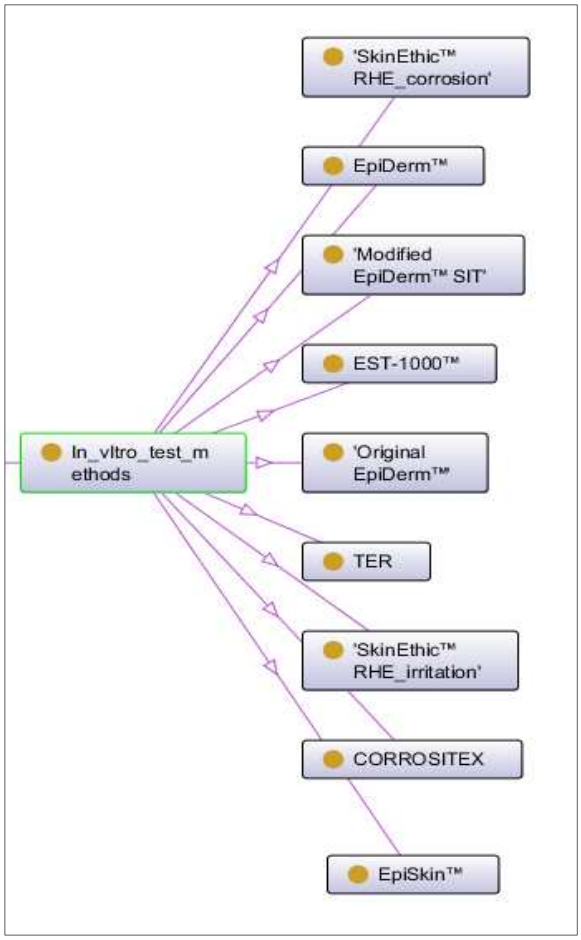


Figure 14. WP1.Separate sub-class describing new *in vitro* assays.

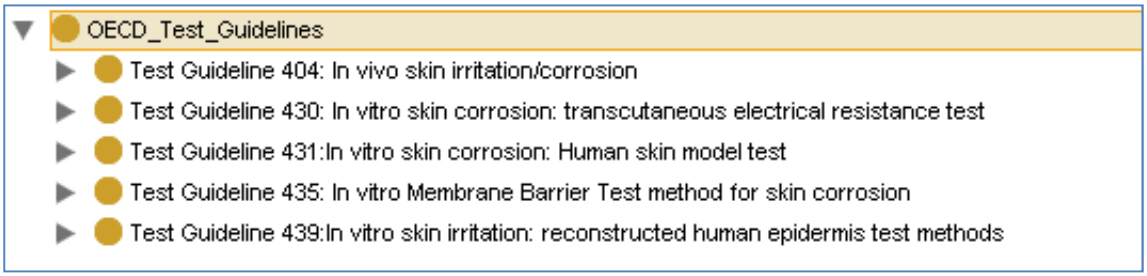


Figure 15. WP1.Separate sub-class describing OECD Test Guidelines.

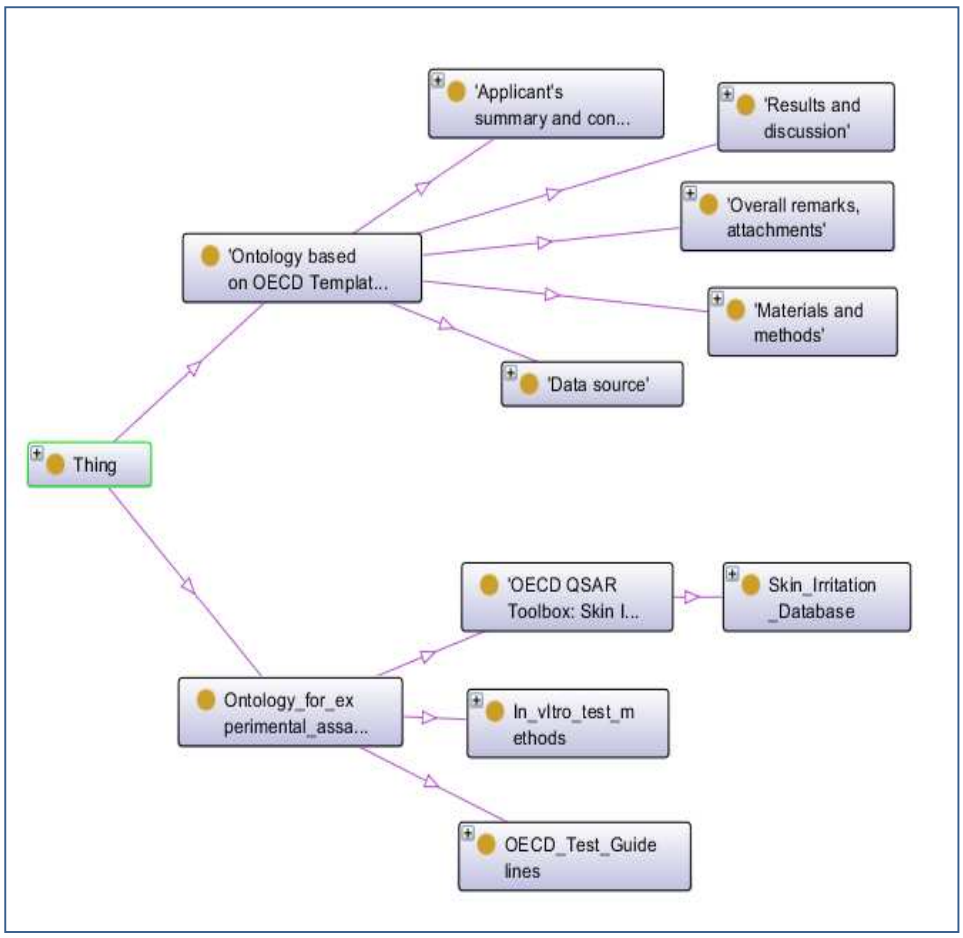


Figure 16. WP1.General structure of the skin irritation/corrosion ontology.

2.3 WP1 Skin irritation/corrosion: Deliverables 2.2 "Compilation of terms related to the endpoint". Detailed work description.

In addition to the terms collected from the QSAR Toolbox datasets additional terms have been collected from different sources including:

- OECD Harmonised Template #64: Skin irritation / corrosion: draft version (Figure 17). Since some important information (OECD fields numbers, picklists numbers, etc) is missing in the draft version, it has been decided to add the prefix "draft" to all term, extracted from the draft HT. This will facilitate the update of the draft terms to the final ones;

-OECD HT #64 free test fields extracted from the HT text documentation (Figure 18);

-OECD Test Guidelines, including TGs covering the new in vitro methods. Additional terms have been found in the Test Guidelines mentioned below and translated into the OWL format (Figure 19);

1. OECD TG 430: in vitro skin corrosion: transcutaneous electrical resistance test
2. OECD TG 431: In vitro skin corrosion: Human skin model test
3. OECD TG 404: In vivo skin irritation/corrosion
4. OECD TG 439: In vitro skin irritation: reconstructed human epidermis test methods
5. OECD TG 435: In vitro Membrane Barrier Test method for skin corrosion

-Additional sources describing the new in vitro assays assays [113] (Figure 20).

Different prefixes have been used for the class label in order to facilitate the further ontology maintenance and possible update:

"Draft:" as explained above for terms, extracted from the draft version of HT;

prefix as a code of the HT picklist, for example, "C08:in vivo" ,where "C08:" is the picklist code for the class "Type of method";

prefix for terms extracted from different OECD Test Guidelines, for example, "TG404: In vivo skin irritation/corrosion", where "TG404:" indicates the TG;

prefix for terms extracted from the OECD QSAR Toolbox databases, for example, "OECD QSAR Toolbox v3 dataset: Reference source".

For some terms the explicit links to the relevant external ontologies have been provided, e.g.

The FMA (Foundational Model Anatomy) (http://sig.uw.edu/fma#Organ) for the class "Organ". Other externals resources that have been used include:

Phenotypic Quality Ontology http://purl.bioontology.org/ontology/PATO;

The NCBITaxon ontology (a taxonomic classification of living organisms and associated artifacts) http://purl.bioontology.org/ontology/NCBITAXON;

The EFO (Experimental factors ontology) http://www.ebi.ac.uk/efo/EFO_0000433;

Units of Measurement Ontology http://bioportal.bioontology.org/ontologies/UO.

Overall about 1200 terms have been implemented in the skin irritation/corrosion ontology.

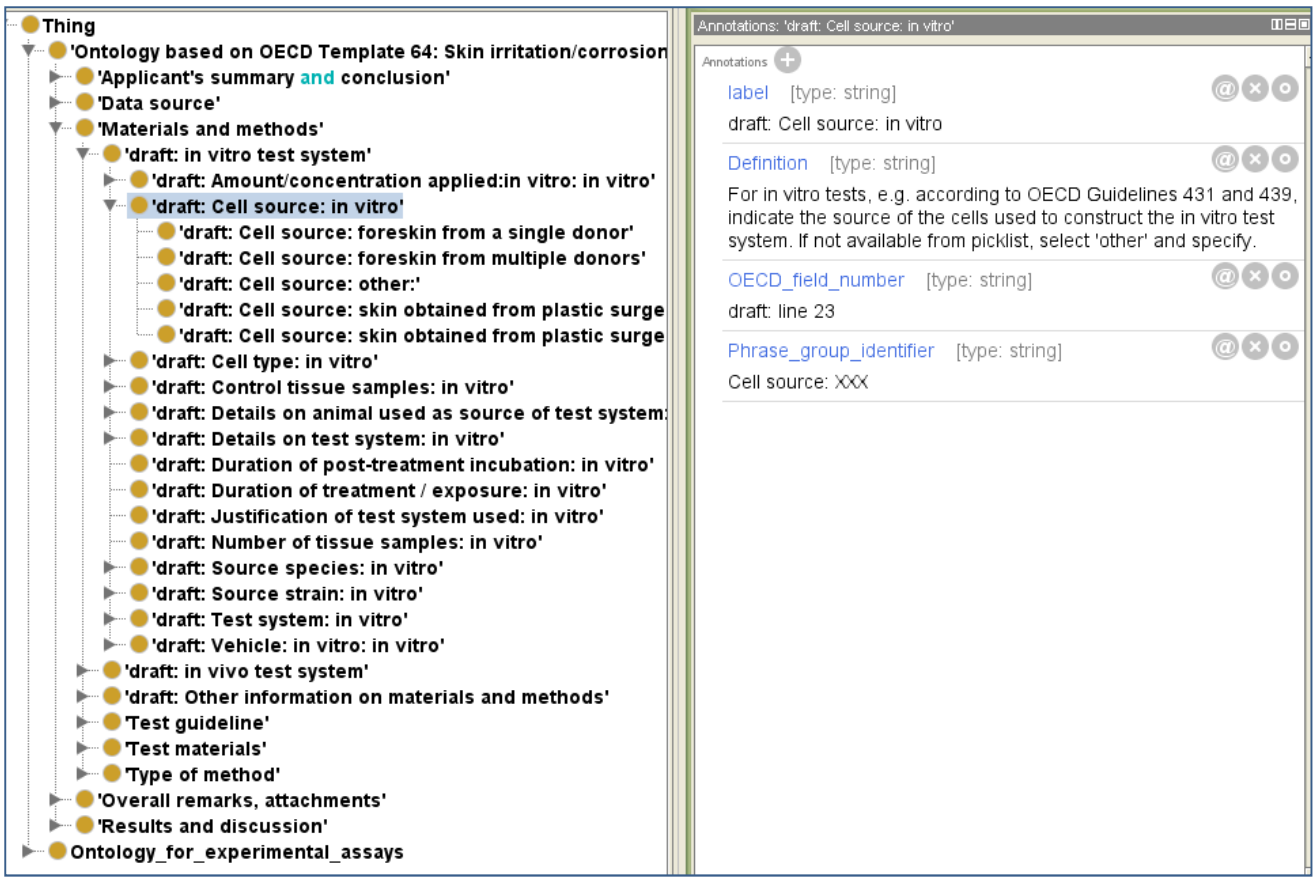


Figure 17. WP1.Example of translation of the draft HT version into the OWL format.

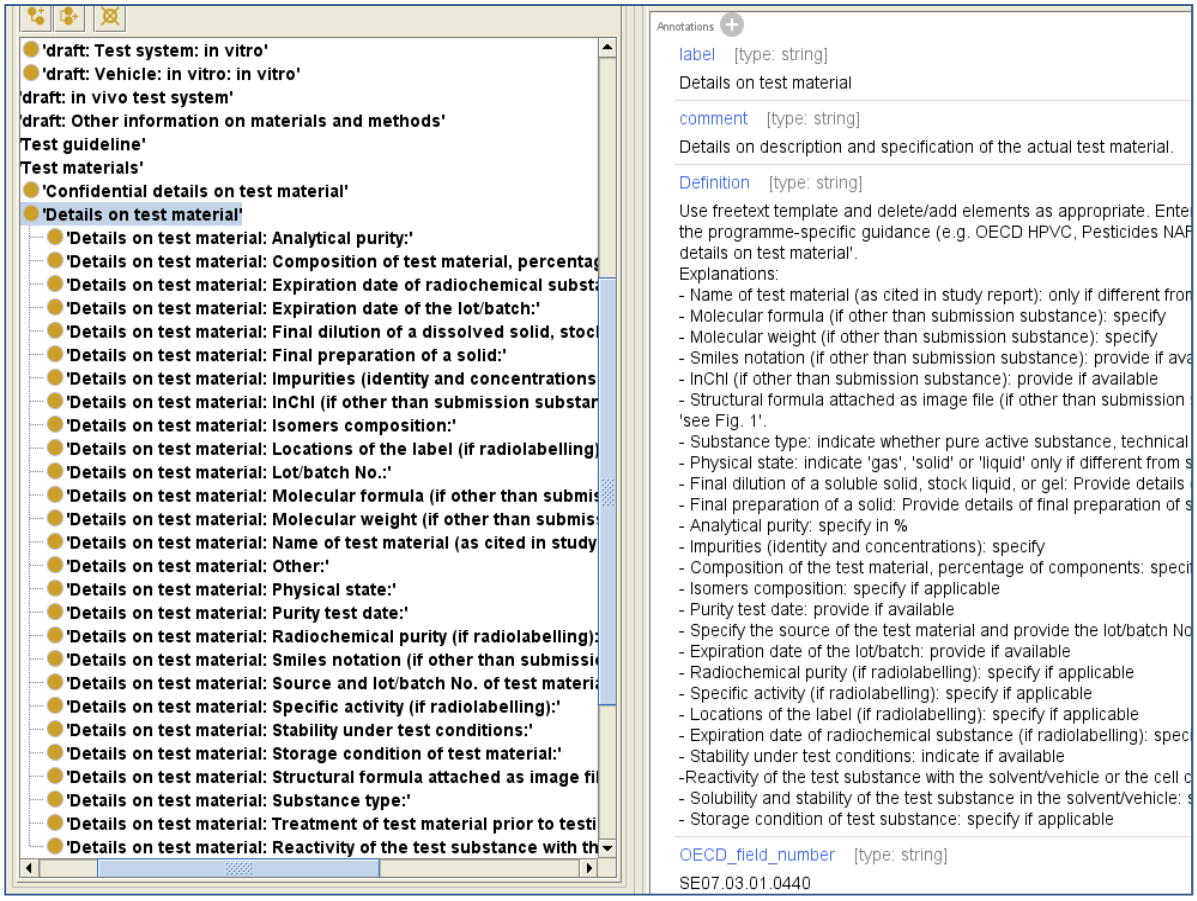


Figure 18. WP1.Example of translation of the free text definition in the OWL classes.

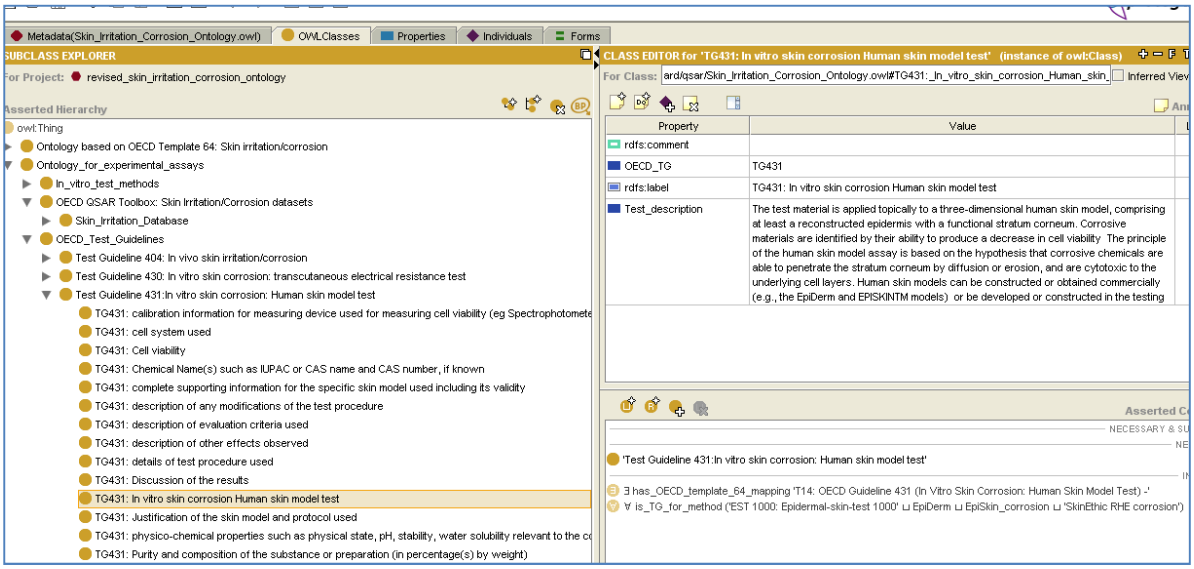


Figure 19. WP1.Example of the ontological description of the OECD Test Guidelines.

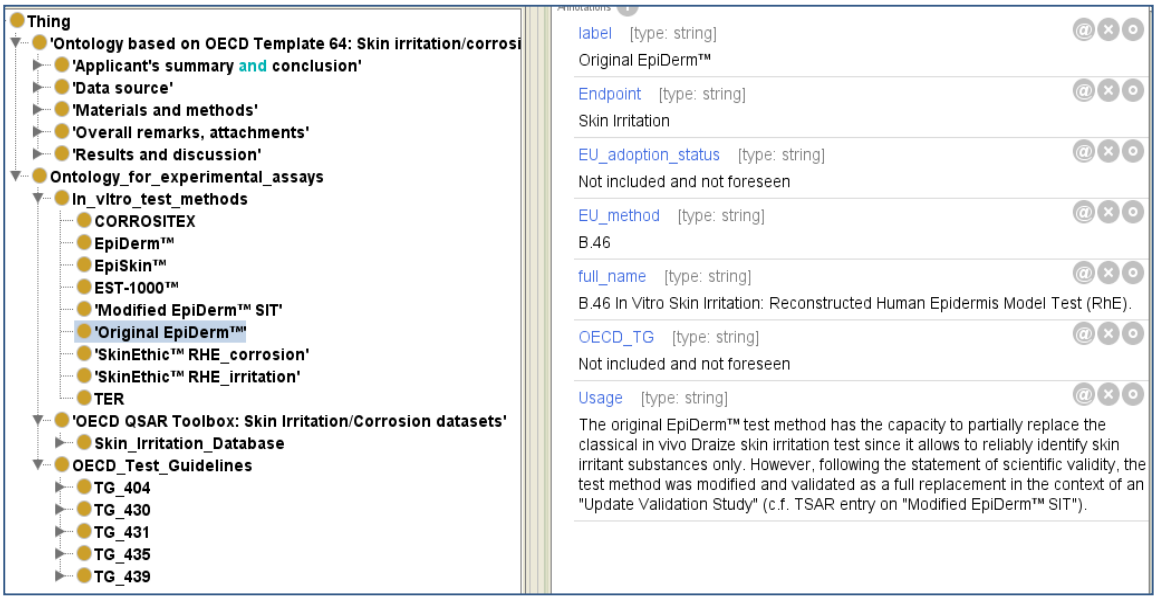


Figure 20. WP1.Example of definition of the in vitro assays.

2.4 WP1 Skin irritation/corrosion: Deliverables 2.3 "Definition of synonyms and homonyms". Detailed work description.

Synonyms and homonyms collection is needed for understanding how the same terms are defined and annotated in different sources, e.g. in the different datasets, the OECD HTs, the external biomedical ontologies. The OHTs terms, if available, are used as standard names.

The synonyms definition for single terms has been performed by introducing the OWL annotation property: "Synonym", using the multiple annotations of type Synonym, containing just one synonym string. An example of such synonyms definition are shown on Figure 21.

Synonyms have been added both manually and automatically when imported from the external resource using the Protégé Bioportal Import plug-in..

OECD_TG	TG404
rdfs:label	TG404: Dermal irritation
Synonym	epidermal necrolysin
Synonym	rubrefacient
Synonym	irritation of skin
Synonym	irritaton skin
Synonym	skin irritation
Test_description	Dermal irritation is the production of reversible damage of the skin following the application of a test substance for up to 4 hours.

rdfs:isDefinedBy	Primary Irritation Index (PI): Dermal irritation is the production of reversible inflammatory changes in irritation skin following the application of a substance. The skin irritation potential is described by the Primary Irritation Index (PII), calculated from erythema and oedema grades based on experimental rabbits. The maximum PII is 8 and the minimum is 0. The grading scale for irritant effects on rabbit skin were originally proposed by Draize and adopted by the OECD (Test Guideline 404) and the US and EU regulatory agencies. The PII can be calculated as: PII = [SUM(Erythema 24/48/72 h)+SUM(Oedema24/48/72 h)] (3 x no. animals) where Erythema is redness of skin produced by vascular congestion or increased perfusion, and Oedema is the presence of abnormally large amounts of fluid in the intercellular tissue spaces of the epidermis, dermis or subcutaneous tissues.
rdfs:label	Primary_Irritation_Index
Synonym	DPII
Synonym	PII
Synonym	Dermal Primary Irritaton Index
Synonym	Primary Irritation Score

Figure 21. WP1.Examples of synonym definition using the objet property "Synonym".

2.5 WP1 Skin irritation/corrosion: Deliverables 2.4 Establishment of relationships, interactions and hierarchies between classes, objects and numerical properties for each term and rules when existing (internal rules and restriction rules).

In OWL language the relationships between ontological classes, internal and restriction rules could be introduced using the properties. The OWL language has three properties' types: object, annotation and datatype.

Object properties define the relationships between two classes and they are distinct from annotation and datatype properties. The role of annotation properties is to provide additional information (definitions, comments, etc) about ontology entities. Annotation properties do not participate in structural inferencing or reasoning. The role of datatype properties is to define the allowable data types and values for data values related to classes.

The Figure 22 shows the full list of object properties created during the skin irritation/corrosion ontology development. For selected property "has_OECD_template_64_mapping" the textual definition (IsDefinedBy) as well as the corresponding domain and range are also shown (Figure 22).

The textual definitions for each property have been add to the OWL using the inbuilt property "IsDefineBy". Ranges and domains have been defined in OWL for all object properties.

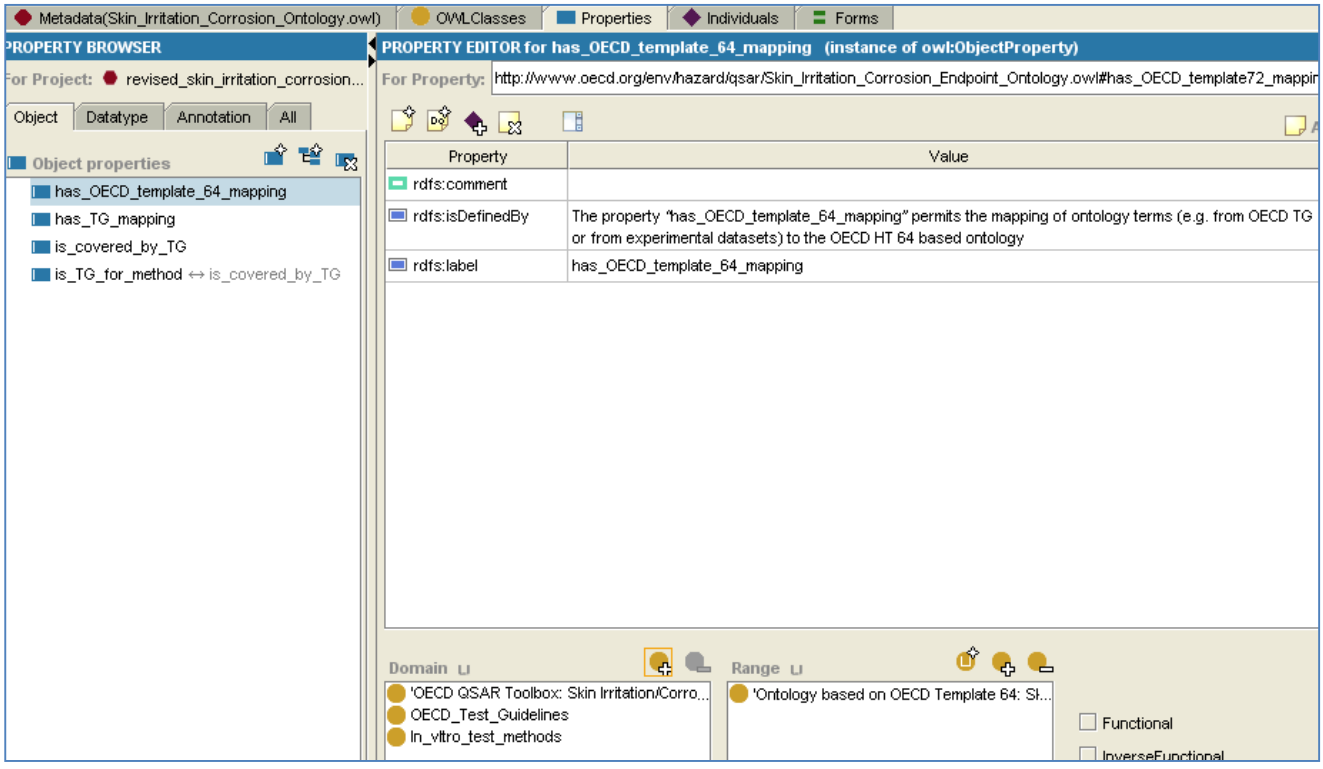


Figure 22. WP1.Protégé Screenshot: object properties created during the skin irritation/corrosion ontology development.

For example, the property ""is_covered_by_TG" and the relative inverse property "is_TG_for_method" allow to stabilize relationship between in vitro test methods and corresponding OECD test guidelines. In the same time

the property “has_OECD_template_64_mapping” allows mapping of ontology terms (e.g. OECD TG) to the OECD HT 64 based ontology (Figure 23).

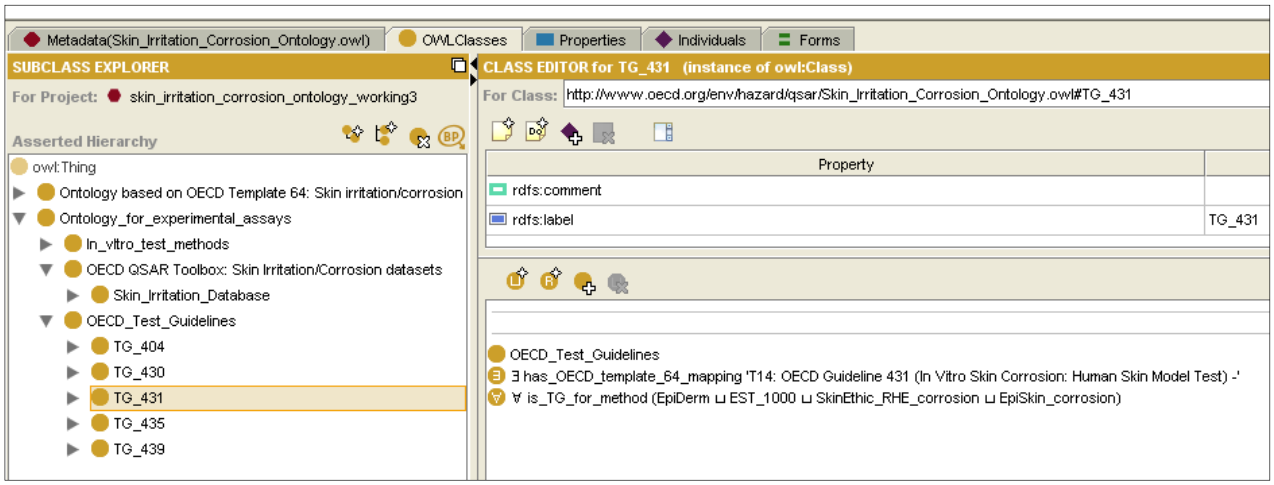


Figure 23. WP1.Protégé Screenshot: example of test guideline terms related to the corresponding test methods and mapped to the HT 64 based ontology.

The terms of the Skin Irritation dataset (the only one experimental database available for the moment in the OECD Toolbox v.3x) have been mapped both to the OECD HT 64 based ontology and when is possible to the corresponding OECD TG 404 terms using the property “has_TG_mapping” (Figure 24).

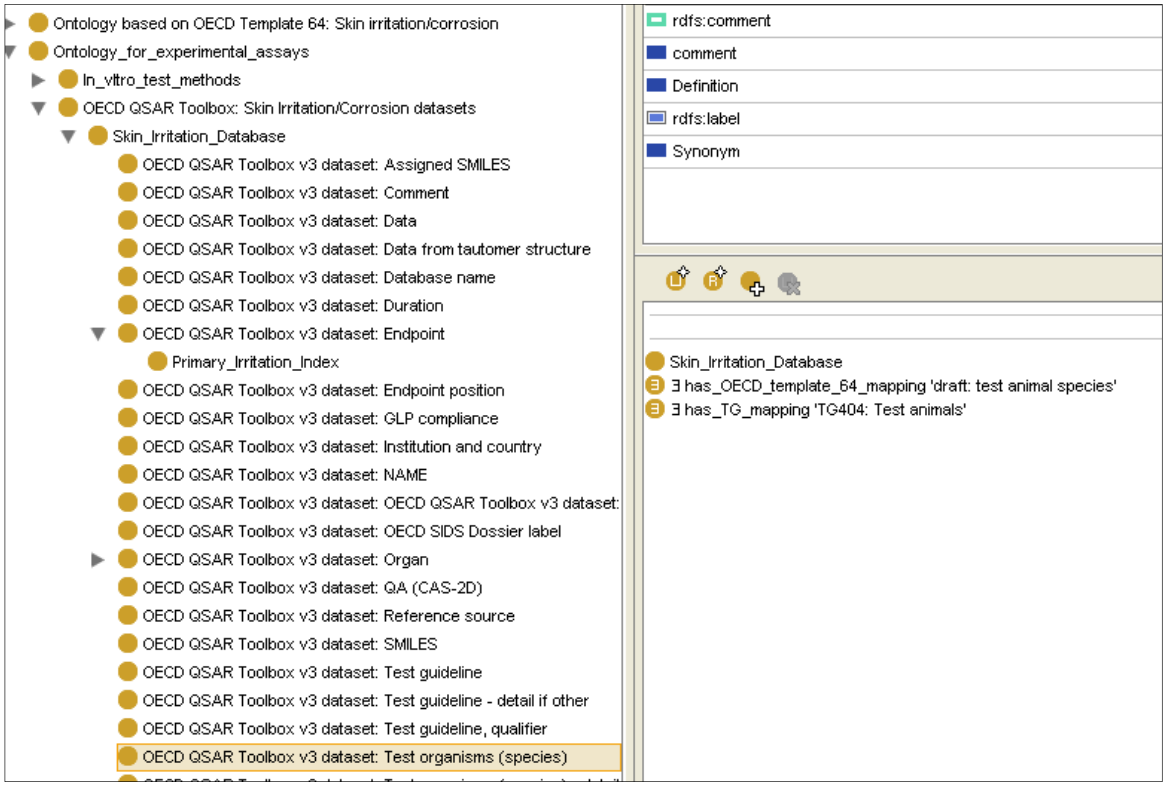


Figure 24. WP1.Protégé Screenshot: example of mapping of experimental dataset’s term to the OECD HT and OECD TG ontology.

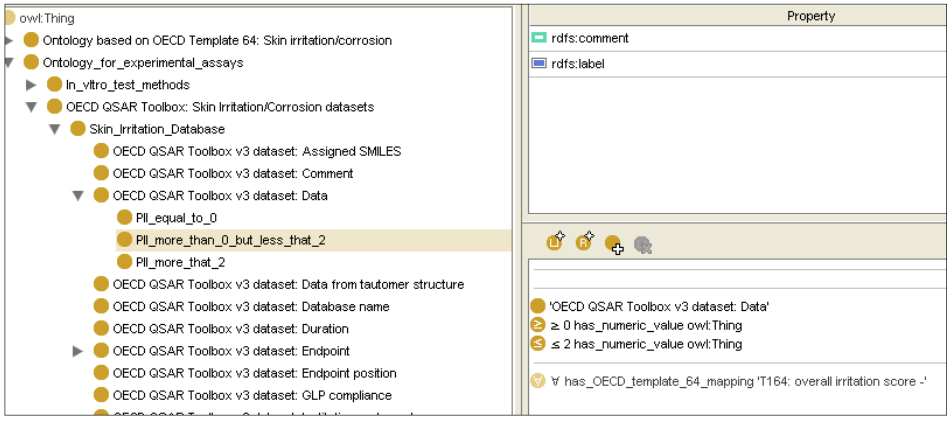


Figure 25. WP1.Protégé Screenshot: example of numeric property use.

The numeric properties are more simple and have been used much less that the object properties, e.g. the property “has_numeric_value” has been used to divide the Primary Irritation Index (PII) in three groups like it is done in the Skin Irritation database (Figure 25).

The ontology metadata have been added using the Inbuilt Protégé annotation properties: “ontologyName”, “created_by” and “ontology version” (Figure 26)

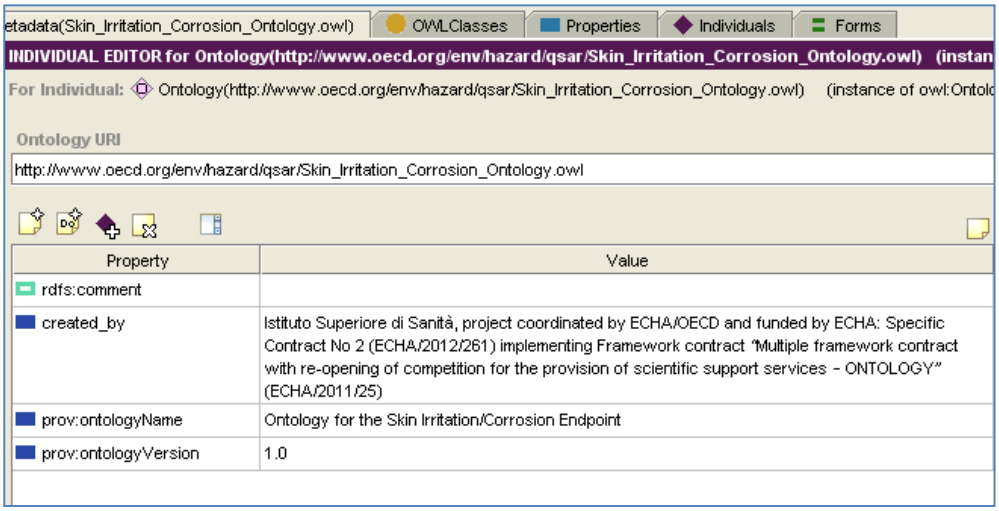


Figure 26. WP1.Protégé Screenshot: ontology metadata.

The full list of the properties (object, annotation and datatype) and textual explanation for each property are shown in the Table 7.

Property	Type of Property	Definition
has_OECD_template_64_mapping	Object	The property "has_OECD_template_64_mapping" permits the mapping of ontology terms (e.g. from OECD TG or from experimental datasets) to the OECD HT 64 based ontology
has_TG_mapping	Object	The property "has_TG_mapping" permits the mapping of ontology terms (e.g. from experimental datasets) to the OECD Test Guidelines terms.
is_covered_by_TG	Object	The property "is_covered_by_TG" and the relative inverse property "is_TG_for_method" permits the stabilization of the relationship between experimental test methods and the corresponding OECD test guidelines.
is_TG_for_method	Object	The property "is_covered_by_TG" and the relative inverse property "is_TG_for_method" permits the stabilization of the relationship between experimental test methods and the corresponding OECD test guidelines.
IsDefinedBy	Annotation	Inbuilt Protégé property used for definition of classes
Comment	Annotation	Inbuilt Protégé property used for definition of classes
Endpoint	Annotation	Indicates the toxicological endpoint for which the method is used
EU_adoption_status	Annotation	Status of OECD adoption for the method
EU_method	Annotation	Indicates the EU protocol for the method described
full_name	Annotation	Full method's name
OECD_field_number	Annotation	Field number imported from the OECD Harmonised template
OECD_TG	Annotation	OECD_TG
Phrase_group_id	Annotation	Number of the corresponding OECD picklist
Phrase_id	Annotation	OECD numeric code for the corresponding picklist
Synonym	Annotation	Synonyms collected for the term
Test_description	Annotation	Brief test description
Usage	Annotation	Indicates the toxicological endpoint for which the method is used
created_by	Annotation	Defines ontology's metadata, inbuilt Protégé property
creation_date	Annotation	Defines ontology's metadata, inbuilt Protégé property
ontologyName	Annotation	Defines ontology's metadata, inbuilt Protégé property
ontologyVersion	Annotation	Defines ontology's metadata, inbuilt Protégé property
has_numeric_value	Datatype	The property allows to specify allowed data values for some numeric terms (number of animals, duration, etc).
has_datatype	Datatype	The property allows to specify allowed data types for some numeric terms (number of animals, duration, etc).

Table 7. *WP1*Complete list of properties used in the OWL file

2.6 Conclusions

The Final Report and the Final version of the Ontology (OWL file in Protégé software) have been prepared for WP1 Skin irritation/corrosion after implementation of Deliverables 2.1-2.5.

The first main class of Skin irritation/corrosion ontology is based on OECD HT # 64 and has five main sub-classes: Overall remarks, attachments, Applicant's summary and conclusion, Data source, Materials and Methods, Results and Discussion. The OECD field number and other information contained in the HTs documentation have been completely translated into the OWL format. Two other main classes includes OECD

The second main class covers QSAR Toolbox Skin Irritation database vocabulary, in vitro assays and relative test guidelines terminology (Deliverable 2.1).

After the terms (Deliverable 2.2). and synonyms (Deliverable 2.3) collection have been finished, the ontology has been completed introducing relationships between classes using the OWL properties and restriction rules (Deliverable 2.4).

3. WP2 Development of ontology for the eye irritation endpoint

3.1 Background

The goal of the WP2 is to develop ontologies suitable for standardizing and organizing the chemical toxicological databases in the OECD (Q)SAR Toolbox [2] for the eye irritation endpoint. The Open Biomedical Ontologies Foundry [3] principles, the Web Ontology Language (OWL) and the Protégé software are used for the implementation.

During the Inception Phase of the Project (Deliverable Report 1) we have identified a number of related resources. A number of different tasks and subtasks have been identified as well.

The most important standard that should be preserved during the ontology implementation is the OECD Harmonized Templates (OECD HTs) [102].

The original terminology of OHTs and databases vocabulary should be maintained in order to fulfill the requirements of the contract and to facilitate the implementation in the QSAR Toolbox.

A first requirement of the ontology is obviously the compatibility of the Ontology with the existing terminology used in the OECD (Q)SAR Toolbox and a flexible ontology approach suitable for further possible extensions. Currently the OECD (Q)SAR Toolbox version 3.0 [2] contains only one eye irritation database that includes experimental results on rabbit eye irritation from ECETOC [10] report Eye Irritation Reference Chemicals Data Bank [34] for 128 chemicals.

As reported during the Inception Phase, we were not able to identify any new database for the eye irritation/corrosion endpoint (e.g. generated with the novel in vitro assays described in this section) in addition to data already available in the OECD Toolbox v. 3.0.

The Organisation for Economic Co-operation and Development (OECD) has adopted a tiered approach for eye irritation, described in the revised TG 405 Acute Eye Irritation/Corrosion (revised in 2012) [116]. TG 405 recommends the use of validated in vitro or ex vivo methods when appropriate. These assays [113] are described in TG 437: Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants [117] and in TG 438: Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants [118]; TG 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants [119] and Draft TG: The Cytosensor Microphysiometer (CM) Test Method: An in vitro Method for Identifying Ocular Corrosive and Severe Irritant Chemicals as well as Chemicals not Classified as Ocular Irritants [120].

We will develop vocabulary/ontology for the new in vitro assays despite the absence of actual database which may be further considered in the future once data become available. This will be like a separate ontology class with specific annotation to enable distinction from the ontology of the present Toolbox databases and will contain names of assays and other available information on the assay as such.

3.2 WP2 Eye irritation: Deliverables 3.1 "Definition of classes and hierarchical relationships in the ontology structure". Detailed work description.

During the WP2 D3.1 implementation we have identified and defined classes and hierarchical relationships for the eye irritation.

The present ontology has to respect regulatory needs, and is tailored on existing constraints, i.e.,: a) the OECD HTs; and b) the eye irritation database in the Toolbox. The goal of the project is to support and improve data integration and mapping in Toolbox, which is very domain-specific task, with most terms/concepts already having an official terminology (used e.g., in the OECD HTs).

To guarantee the compatibility of ontology with the OECD QSAR Toolbox, the ontology was separated into two main super-classes: the first one is dedicated to HT # 65 Eye irritation, while the second covers the experimental datasets, OECD test guidelines and novel in vitro assays terminology. Using this approach, it is possible to add smoothly any new eye irritation/corrosion dataset to the ontology.

The most important standard that has been preserved during the ontology implementation is the OECD Harmonized Templates (OECD HTs) [102] both official and draft versions. We have conserved all documentation and the structure of the OECD Harmonised Template #65: Eye irritation [102] and relative picklists [104].

Since some important information (OECD fields numbers, picklists numbers, etc) is missing in the draft version, it has been decided to add the prefix "draft" to all the terms extracted from the draft HT (Figure 27). This will facilitate the update of the draft terms to the final ones.

The conversion of the HT 65 into the OWL format has been performed semi-automatically, with the Protégé Excel Import Plug-in. The ontology class based on OECD HT # 65: has five main sub-classes: Overall remarks, attachments; Applicant's summary and conclusion; Data source; Materials and Methods; Results and Discussion. The OECD field number and other information contained in the HTs documentation was maintained in order to facilitate the ontology maintenance in case of HTs updates.

When possible we have imported the SC1 Ontologies parts mostly for the overlapping administrative terms of the HT. The rest of work has been done from scratch.

The second part of the ontology "Ontology for experimental assays" covers

- the eye irritation database from the OECD QSAR Toolbox software v.3,

-new in vitro assays despite the absence of actual database, which can be further considered once date become available,

-OECD Test Guidelines for eye irritation in vivo and in vitro.

The eye irritation database is included in the OECD (Q)SAR Toolbox v. 3.0. The general structure of the databases has been translated in OWL format (Figure 28). This dataset will be further mapped onto the OECD HT 65 ontology.

In a careful preliminary survey we did not obtain any information about the existence of new systematic databases, e.g. data generated with novel in vitro assays. However it has been decided to develop vocabulary/ontology for new in vitro assays despite the absence of actual database. We have included the vocabulary for these assays in the separate ontology class "In vitro test methods" (Figure 29). In such a way the ontology concept will be kept flexible and it will be possible to extend the ontology to cover new datasets when and if available in the next future.

The subclass "OECD Test Guidelines" covers the following Test Guidelines for eye irritation (Figure 30):

TG 405: Acute Eye Irritation/Corrosion;

TG 437: Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants;

TG 438: Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants;

TG 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants;

Draft TG: The Cytosensor Microphysiometer (CM) Test Method: An in vitro Method for Identifying Ocular Corrosive and Severe Irritant Chemicals as well as Chemicals not Classified as Ocular Irritants.

The general structure of the eye irritation ontology is shown in Figure 31.

The terms collection for free text fields of the HT, eye irritation database and for OECD test guidelines will be completed during the D 3.2 implementation.

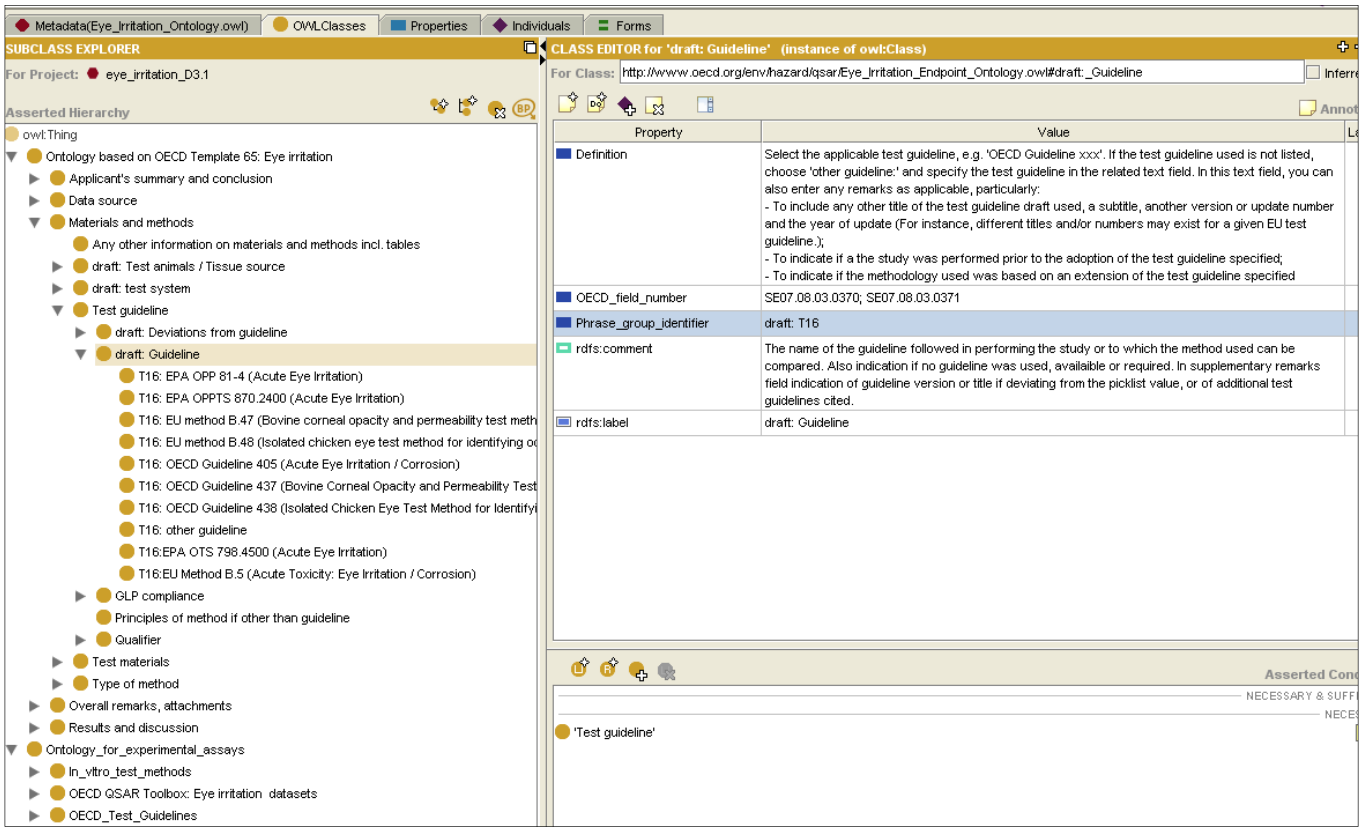


Figure 27. WP2.Protégé Screenshot: the HT's fragment in OWL format.

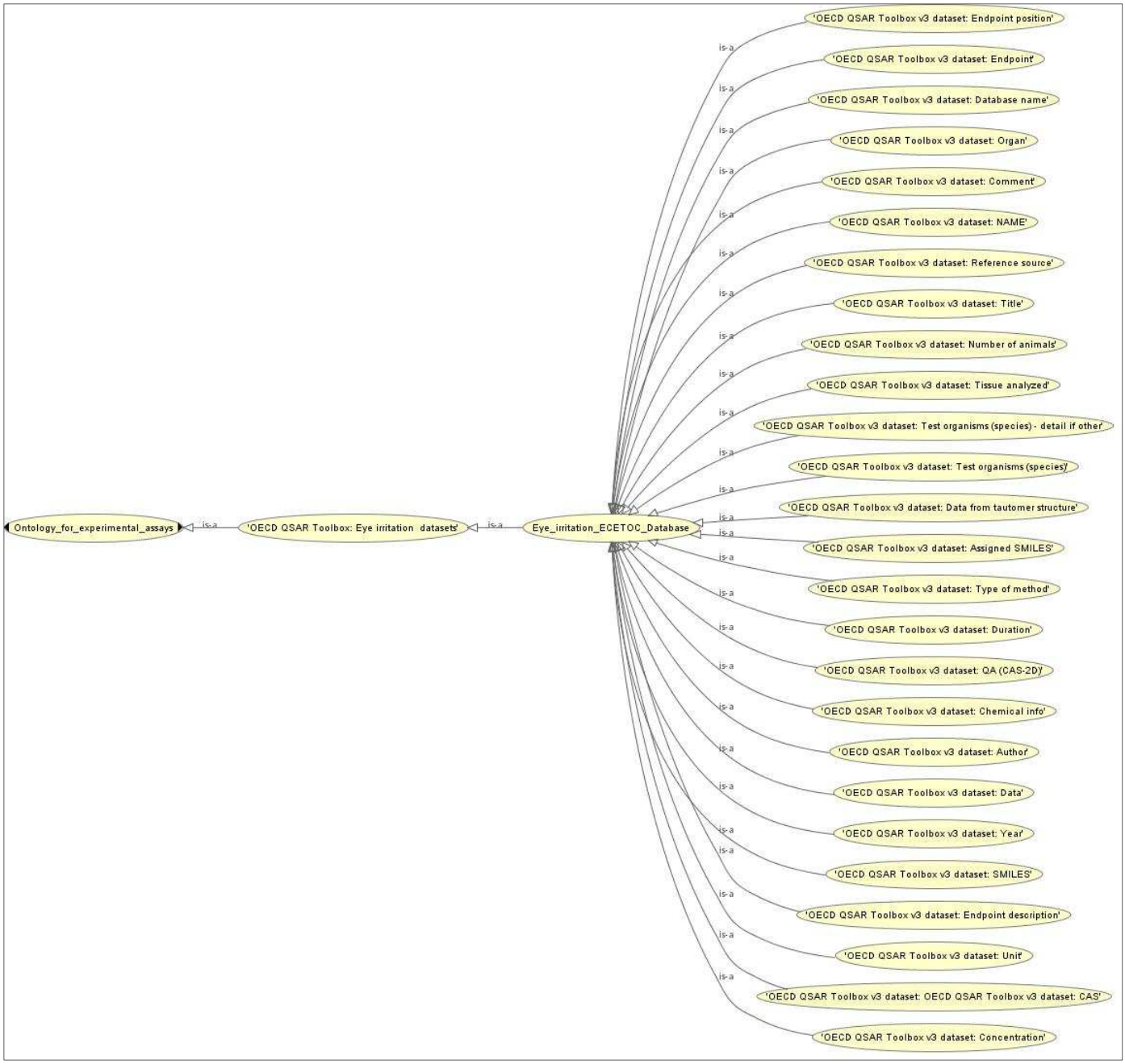


Figure 28. WP2.Main sub-classes describing the eye irritation database.

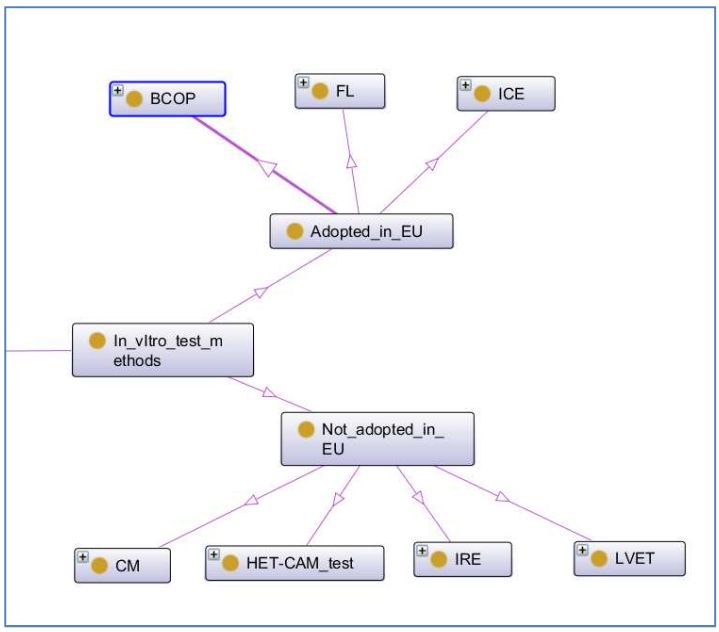


Figure 29. WP2.Separate sub-class describing new *in vitro* assays.

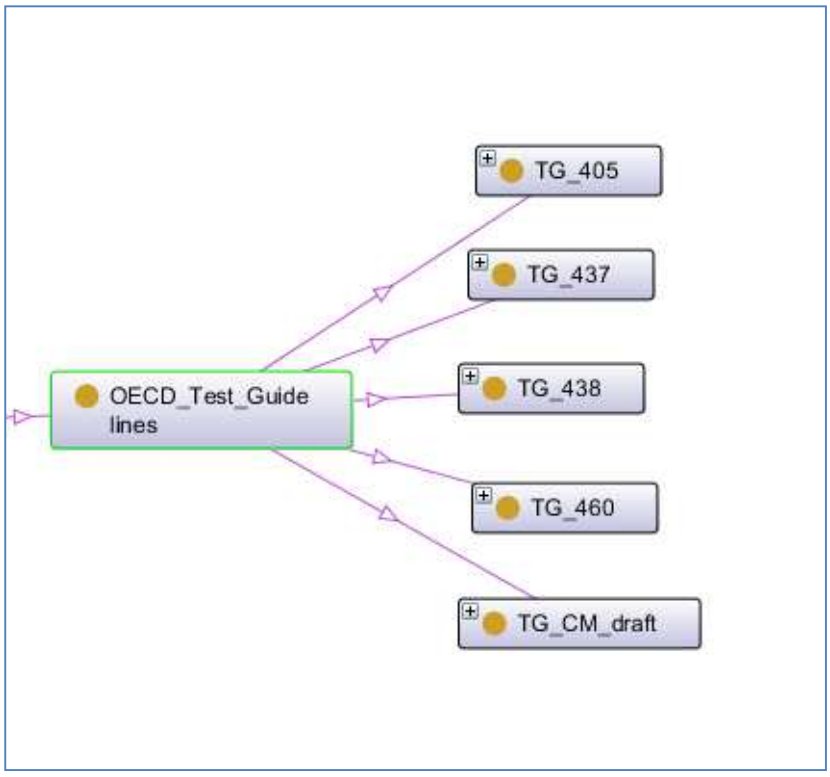


Figure 30. WP2.Separate sub-class describing OECD Test Guidelines.

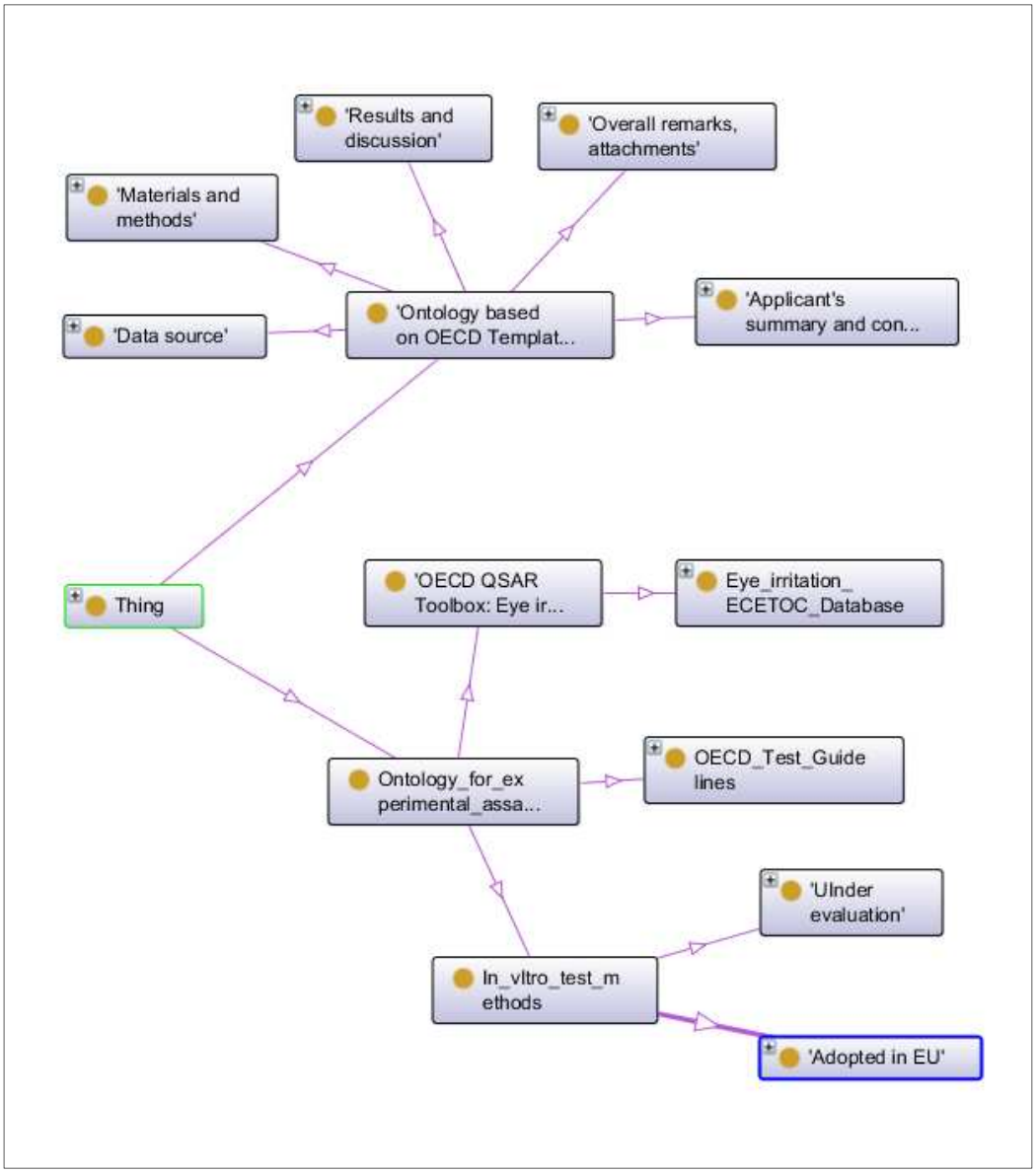


Figure 31. WP2.General structure of the eye irritation ontology.

3.3 WP2 Eye irritation: Deliverables 3.2 "Compilation of terms related to the endpoint". Detailed work description.

In addition to the terms collected from the QSAR Toolbox datasets additional terms have been collected from different sources including:

- OECD Harmonised Template #65: Eye irritation: draft version (Figure 32). Since some important information (OECD fields numbers, picklists numbers, etc) is missing in the draft version, it has been decided to add the prefix "draft" to all term, extracted from the draft HT. This will facilitate the update of the draft terms to the final ones;

-OECD HT #65 free test fields extracted from the HT text documentation (Figure 33);

-OECD Test Guidelines, including TGs covering the new in vitro methods. Additional terms have been found in the Test Guidelines mentioned below and translated into the OWL format (Figure 34):

TG 405: Acute Eye Irritation/Corrosion;
TG 437: Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants;
TG 438: Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants
TG 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants;

Draft TG: The Cytosensor Microphysiometer (CM) Test Method: An in vitro Method for Identifying Ocular Corrosive and Severe Irritant Chemicals as well as Chemicals not Classified as Ocular Irritants.

-Additional sources describing the new in vitro assays assays [113, 121, 122] (Figure 35). For the in vitro assays for which the OECD TGs are available (BCOP, OCE; FL and CM (draft version of TG)), the vocabulares are collected under the corresponding TG class (Figure 34, 35a). In other cases (HET-CAM, IRE, LVET) the vocabularies are collected under the corresponding assay class (Figure 35b).

Different prefixes have been used for the class label in order to facilitate the further ontology maintenance and possible update:

"Draft:" as explained above for terms, extracted from the draft version of HT;

prefix as a code of the HT picklist, for example, "Z31: publication" ,where "Z31:" is the picklist code for the class "Reference type";

prefix for terms extracted from different OECD Test Guidelines, for example, "TG404: In vivo skin irritation/corrosion", where "TG405:" indicates the TG;

prefix for terms extracted from the OECD QSAR Toolbox databases, for example, "OECD QSAR Toolbox v3 dataset: Reference source".

For some terms the explicit links to the relevant external ontologies have been provided, e.g.

The FMA (Foundational Model Anatomy) (http://sig.uw.edu/fma#Organ) for the class "Organ". Other externals resources that have been used include:

Phenotypic Quality Ontology http://purl.bioontology.org/ontology/PATO;

The NCBITaxon ontology (a taxonomic classification of living organisms and associated artifacts) http://purl.bioontology.org/ontology/NCBITAXON;

The EFO (Experimental factors ontology) http://www.ebi.ac.uk/efo/EFO_0000433;

Units of Measurement Ontology http://bioportal.bioontology.org/ontologies/UO.

Overall about 1100 terms have been implemented in the eye irritation/corrosion ontology.

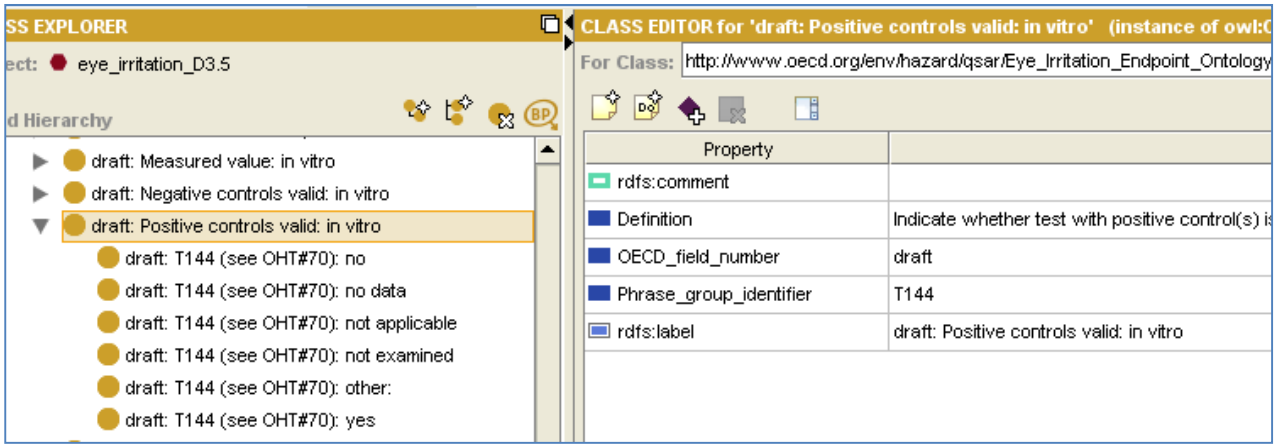


Figure 32. WP2.Example of translation of the draft HT version into the OWL format.

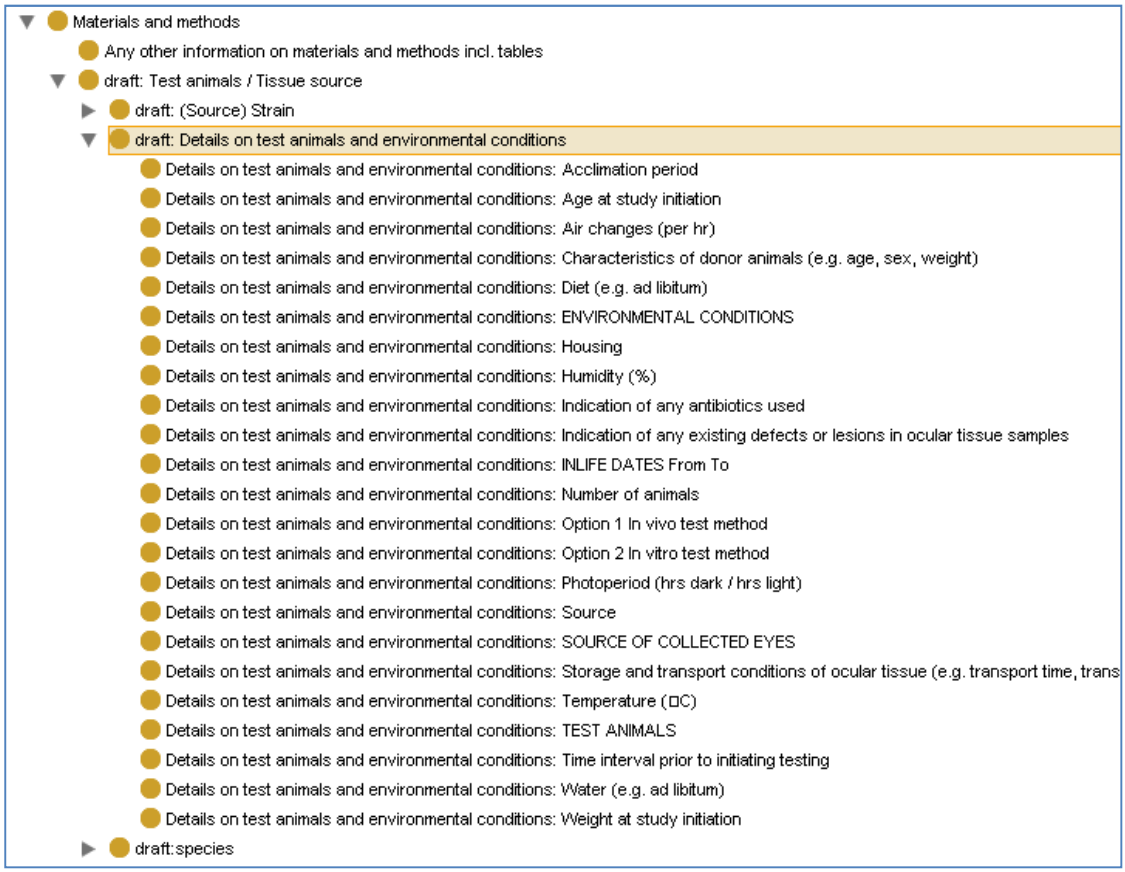


Figure 33. WP2.Example of translation of the free text definition in the OWL classes.

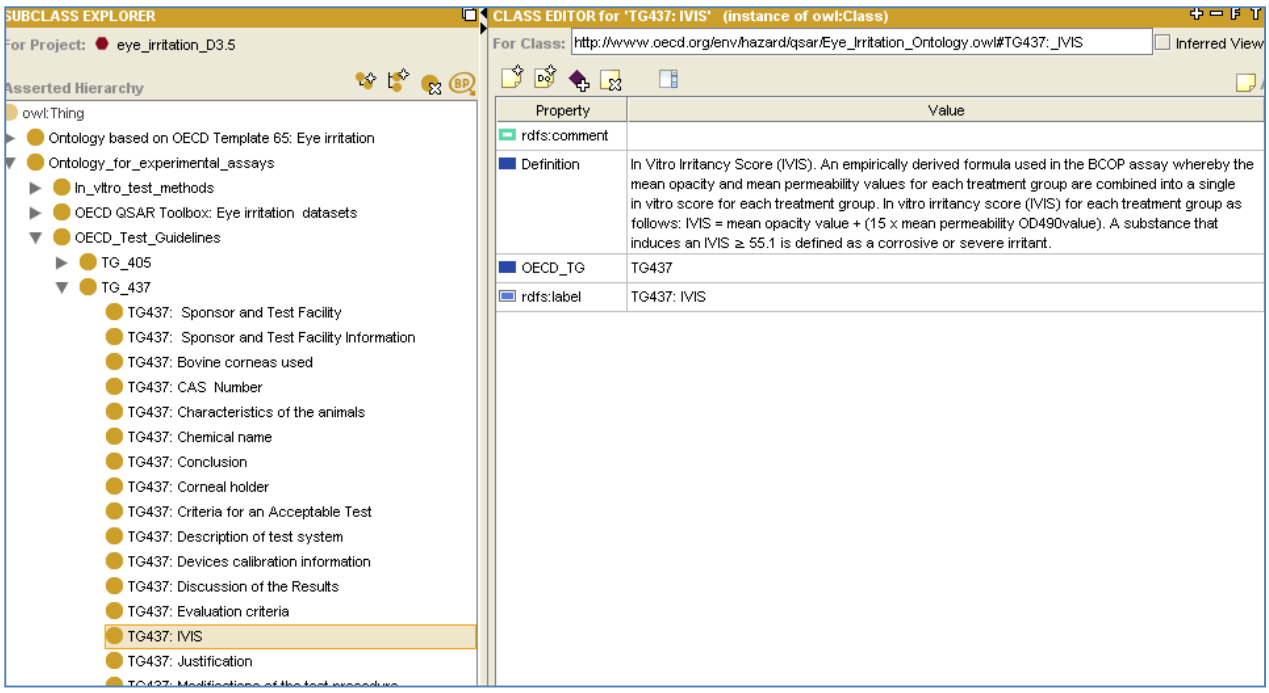
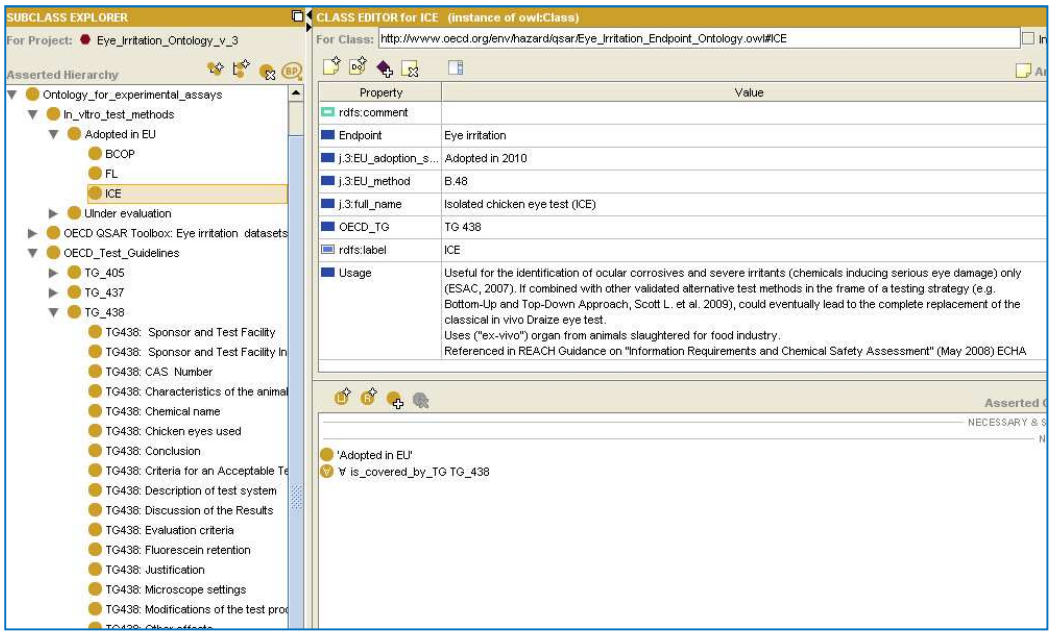
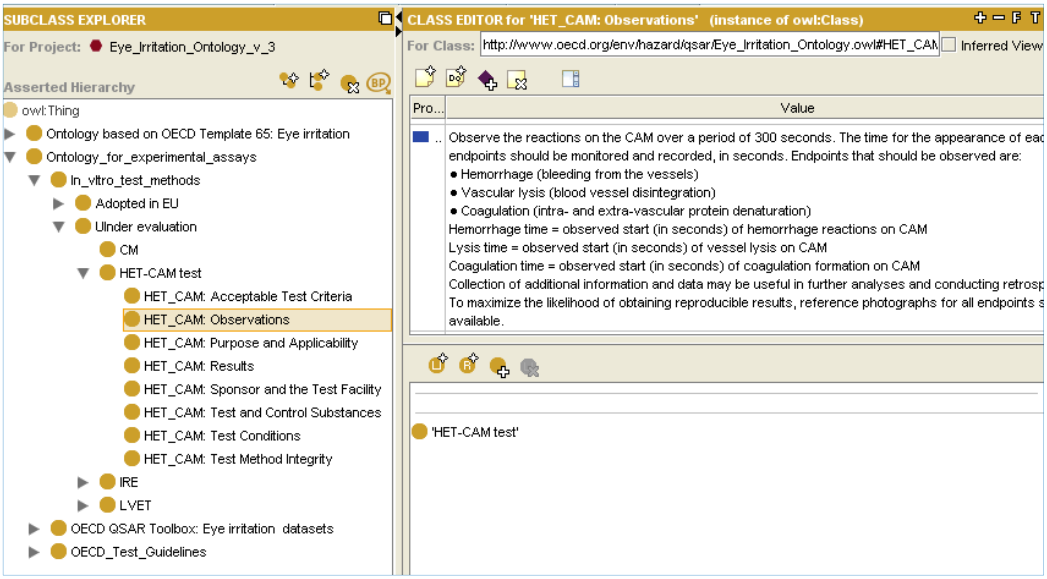


Figure 34. WP2.Example of the ontological description of the OECD Test Guidelines.



a



b

Figure 35. WP2.Examples of definition of the in vitro assays.

3.4 WP2 Eye irritation: Deliverables 3.3 "Definition of synonyms and homonyms". Detailed work description.

The collection of synonyms and homonyms is necessary for understanding how the same terms are defined and annotated in different sources, e.g. in the different datasets, the OECD HTs, the external biomedical ontologies. The OHTs terms, if available, are used as standard names.

The synonyms definition for single terms has been performed by introducing the OWL annotation property: "Synonym", using the multiple annotations of type Synonym, containing just one synonym string. An example of such synonyms definition is shown on Figure 36.

Synonyms have been added both manually and automatically when imported from the external resource using the Protégé Bioportal Import plug-in, which allows the direct Bioportal depository access directly from the Protégé.

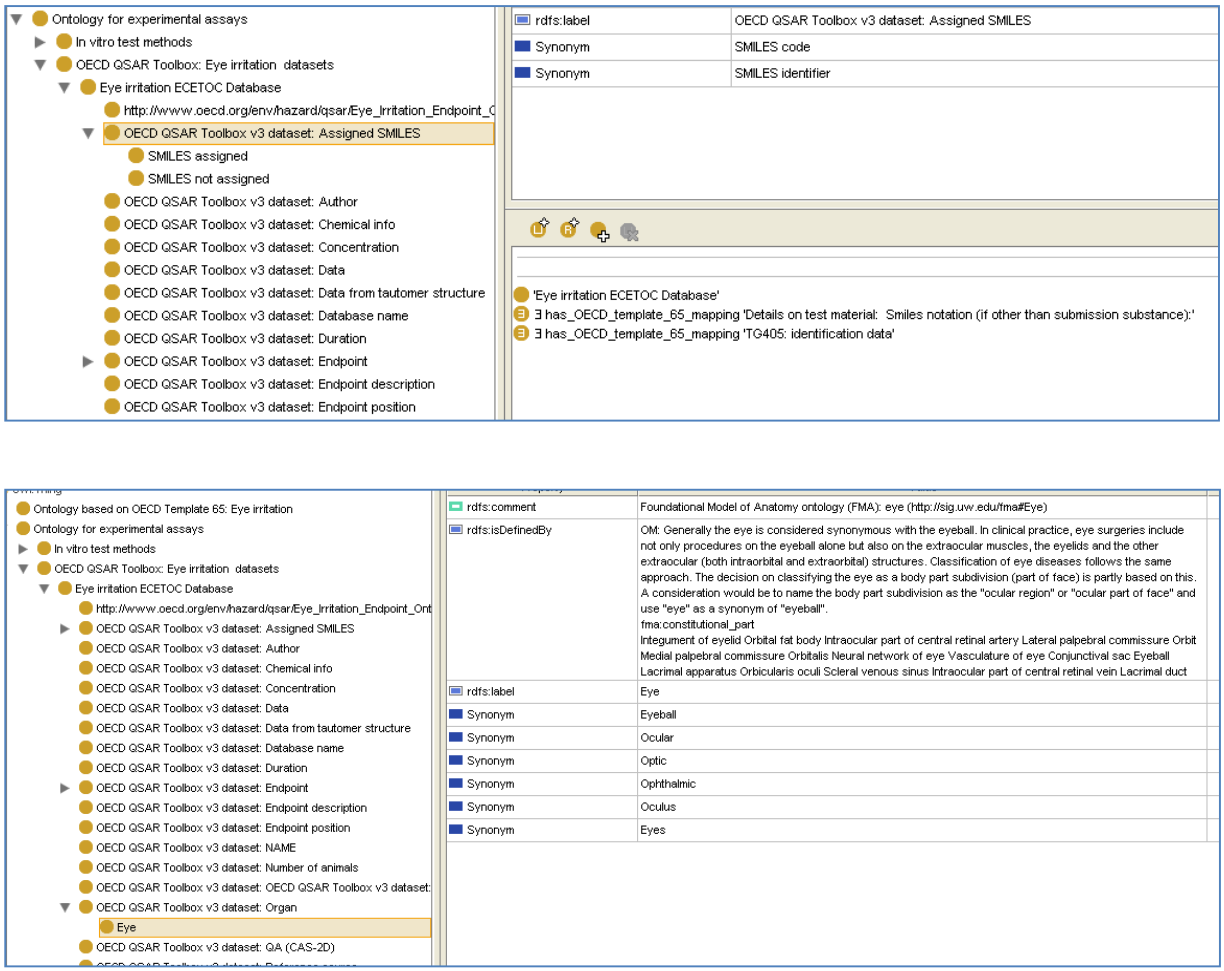


Figure 36. WP2.Examples of synonym definition using the objet property "Synonym".

3.5 WP2 Eye irritation/corrosion: Deliverables 3.4 Establishment of relationships, interactions and hierarchies between classes, objects and numerical properties for each term and rules when existing (internal rules and restriction rules).

In OWL language the relationships between ontological classes, internal and restriction rules could be introduced using the properties. The OWL language has three properties' types: object, annotation and datatype.

Object properties define the relationships between two classes and they are distinct from annotation and datatype properties. The role of annotation properties is to provide additional information (definitions, comments, etc) about ontology entities. Annotation properties do not participate in structural inferencing or reasoning. The role of datatype properties is to define the allowable data types and values for data values related to classes.

The Figure 37 shows the full list of object properties created during the skin irritation/corrosion ontology development. For selected property "has_OECD_template_65_mapping" the textual definition (IsDefinedBy) as well as the corresponding domain and range are also shown (Figure 11).

The textual definitions for each property have been add to the OWL using the inbuilt property "IsDefineBy". Ranges and domains have been defined in OWL for all object properties.

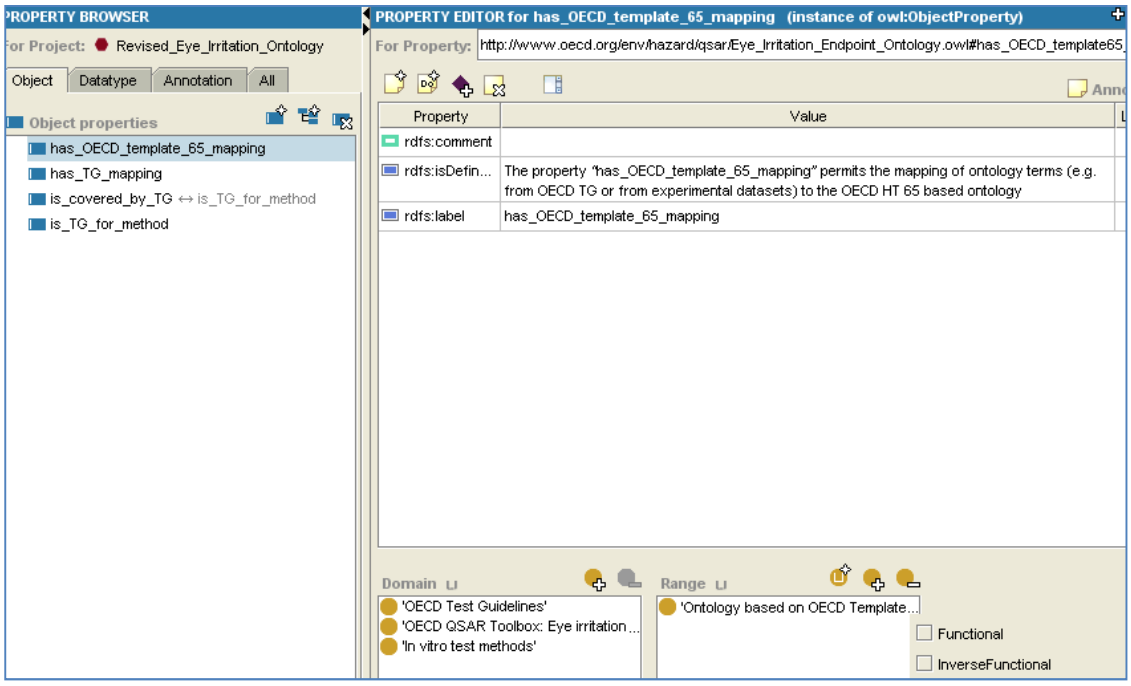


Figure 37. WP2.Protégé Screenshot: object properties created during the eye irritation/corrosion ontology development.

For example, the property "is_covered_by_TG" and the relative inverse property "is_TG_for_method" permits the stabilization of the relationship between in vitro test methods and the corresponding OECD test guidelines. At the same time the property "has_OECD_template_65_mapping" permits the mapping of ontology terms (e.g. OECD TG) to the OECD HT 65 based ontology (Figure 38).

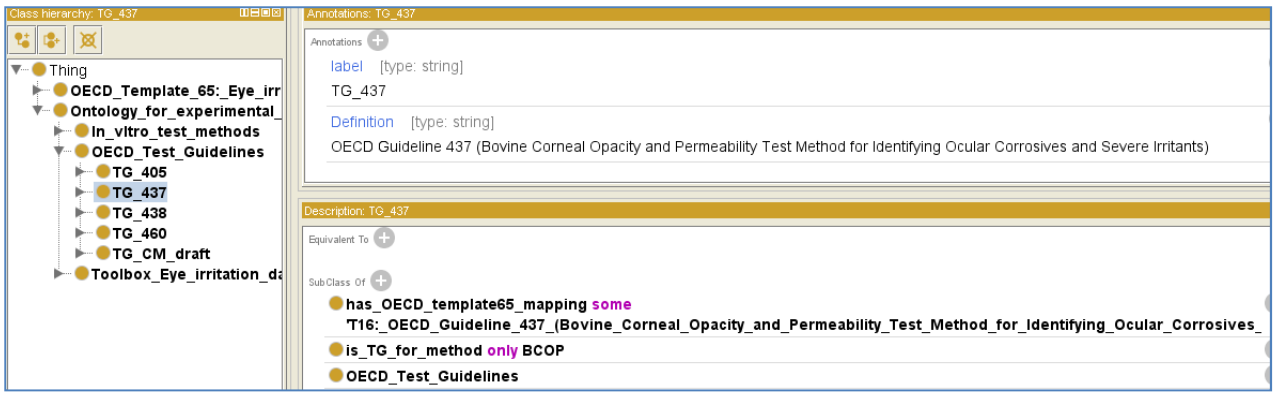


Figure 38. WP2.Protégé Screenshot: example of test guideline terms related to the corresponding test methods and mapped to the HT 65 based ontology.

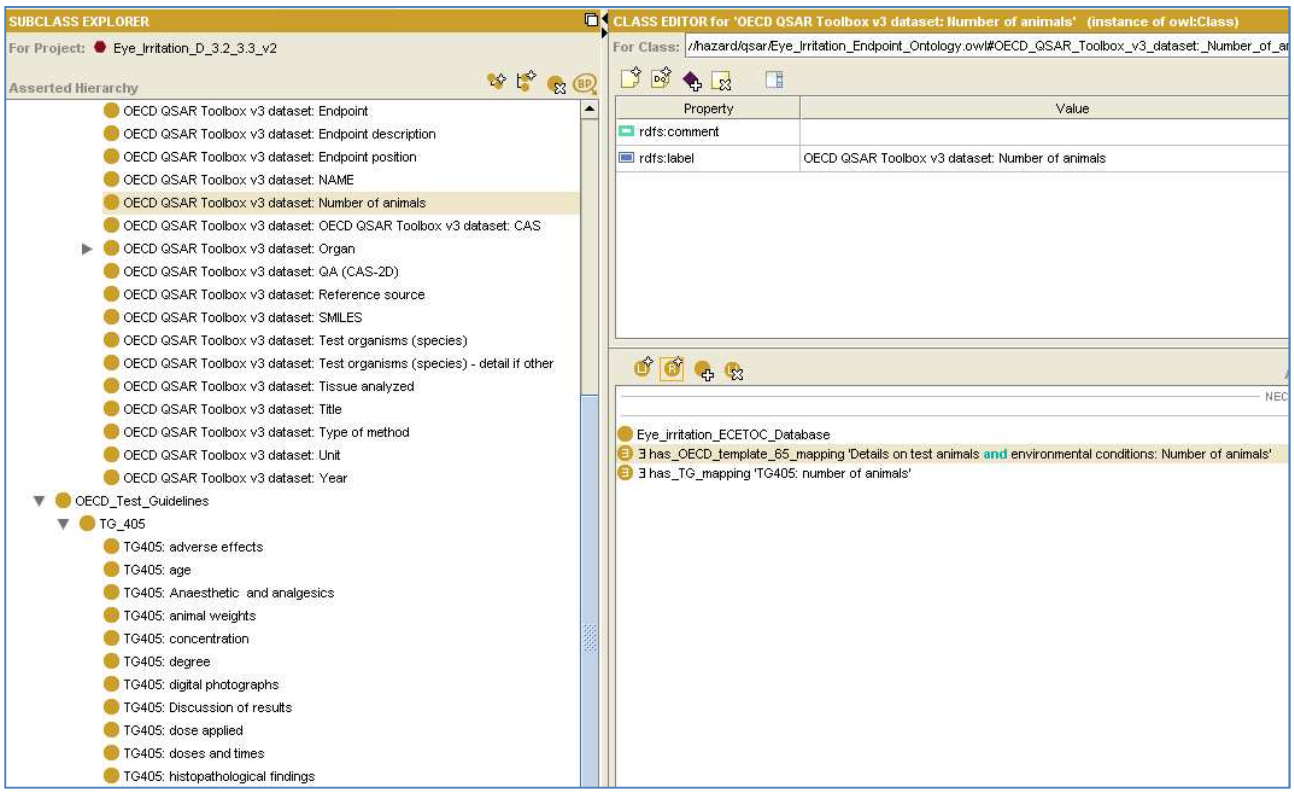


Figure 39. WP2.Protégé Screenshot: example of mapping of experimental dataset's term to the OECD HT and OECD TG ontology.

The terms of the Eye Irritation dataset (the only experimental database available at present in the OECD Toolbox v.3x) have been mapped both to the OECD HT 65 based ontology and, when possible, to the corresponding OECD TG 405 terms using the property "has_TG_mapping" (Figure 39).

The numeric properties are simpler and have been used much less than the object properties, e.g. the property "has_data_type" (integer) has been used to the Test Duration definition in the Eye Irritation database (Figure 40).

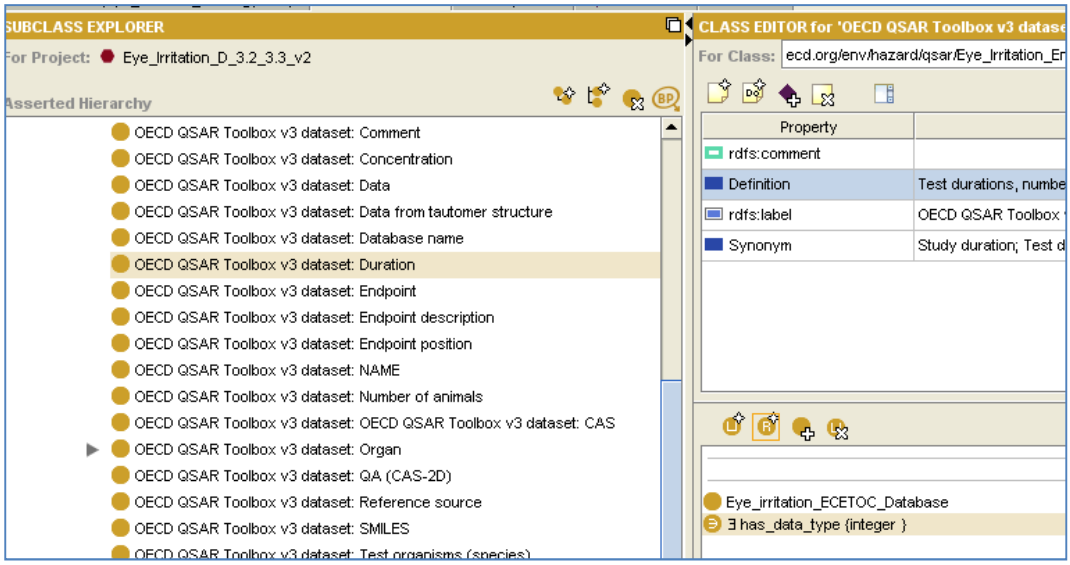


Figure 40. WP2.Protégé Screenshot: example of numeric property use.

The ontology metadata have been added using the Inbuilt Protégé annotation properties: "ontologyName", "created_by" and "ontology version" (Figure 41)

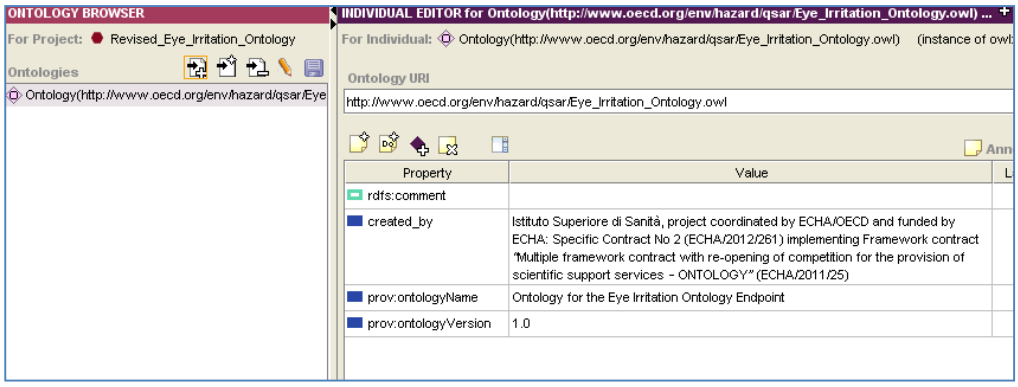


Figure 41. WP2.Protégé Screenshot: ontology metadata.

The full list of the properties (object, annotation and datatype) and textual explanation for each property are shown in the Table 8.

Property	Type of Property	Definition
has_OECD_template_65_mapping	Object	The property "has_OECD_template_65_mapping" permits the mapping of ontology terms (e.g. from OECD TG or from experimental datasets) to the OECD HT 64 based ontology
has_TG_mapping	Object	The property "has_TG_mapping" permits the mapping of ontology terms (e.g. from experimental datasets) to the OECD Test Guidelines terms.
is_covered_by_TG	Object	The property "is_covered_by_TG" and the relative inverse property "is_TG_for_method" permits the stabilization of the relationship between experimental test methods and the corresponding OECD test guidelines.
is_TG_for_method	Object	The property "is_covered_by_TG" and the relative inverse property "is_TG_for_method" permits the stabilization of the relationship between experimental test methods and the corresponding OECD test guidelines.
IsDefinedBy	Annotation	Inbuilt Protégé property used for definition of classes
Comment	Annotation	Inbuilt Protégé property used for definition of classes
Endpoint	Annotation	Indicates the toxicological endpoint for which the method is used
EU_adoption_status	Annotation	Status of OECD adoption for the method
EU_method	Annotation	Indicates the EU protocol for the method described
full_name	Annotation	Full method's name
OECD_field_number	Annotation	Field number imported from the OECD Harmonised template
OECD_TG	Annotation	OECD_TG
Phrase_group_id	Annotation	Number of the corresponding OECD picklist
Phrase_id	Annotation	OECD numeric code for the corresponding picklist
Synonym	Annotation	Synonyms collected for the term
Test_description	Annotation	Brief test description
Usage	Annotation	Indicates the toxicological endpoint for which the method is used
created_by	Annotation	Defines ontology's metadata, inbuilt Protégé property
creation_date	Annotation	Defines ontology's metadata, inbuilt Protégé property
ontologyName	Annotation	Defines ontology's metadata, inbuilt Protégé property
ontologyVersion	Annotation	Defines ontology's metadata, inbuilt Protégé property
has_numeric_value	Datatype	The property allows to specify allowed data values for some numeric terms (number of animals, duration, etc).
has_datatype	Datatype	The property allows to specify allowed data types for some numeric terms (number of animals, duration, etc).

Table 8. WP2.Complete list of properties used in the OWL file

3.6 Conclusions

The Final Report and the Final version of the Ontology (OWL file in Protégé software) have been prepared for WP2 Eye irritation/corrosion after implementation of Deliverables 3.1-3.5.

The first main class of Eye irritation/corrosion ontology is based on OECD HT # 65 and has five main sub-classes: Overall remarks, attachments, Applicant's summary and conclusion, Data source, Materials and Methods, Results and Discussion. The OECD field number and other information contained in the HTs documentation have been completely translated into the OWL format.

The second main class covers the QSAR Toolbox Eye Irritation database vocabulary, the *in vitro* assays and the relative test guidelines terminology (Deliverable 3.1).

After the terms (Deliverable 3.2). and synonyms (Deliverable 3.3) collection have been finished, the ontology has been completed introducing relationships between classes using the OWL properties and restriction rules (Deliverable 3.4).

4. WP3 Development of ontology for the Skin/Respiratory sensitization endpoint

4.1 Background

The goal of the WP3 is to develop ontologies suitable for standardizing and organizing the chemical toxicological databases present in the OECD (Q)SAR Toolbox v. 3 [2] for skin sensitisation. The Open Biomedical Ontologies Foundry [3] principles, the Web Ontology Language (OWL) and the Protégé software are be used for the implementation.

During the Inception Phase of the Project (WP1) we have identified a number of related resources. A number of different tasks and subtasks have been identified as well.

The most important standard that should be preserved during the ontology implementation is the OECD Harmonized Templates (OECD HTs) [102].

The original terminology of OHTs and databases vocabulary should be maintained in order to fulfill the requirements of the contract and to facilitate the implementation in the QSAR Toolbox.

A first requirement of the ontology is obviously the compatibility of the Ontology with the existing terminology used in the OECD (Q)SAR Toolbox and the flexible ontology approach suitable for further possible extensions.

Currently the OECD (Q)SAR Toolbox version 3.1 [2] contains generation of the following databases (DBs) relative to the endpoint to be covered by ontology, as required by the Specific Contract 2:

-skin sensitization;

-skin sensitization ECETOC;

-dendritic cells COLIPA (Adverse outcome pathways);

- keratinocyte gene expression Givaudan (Adverse outcome pathways);

- chemical Reactivity COLIPA (Adverse outcome pathways);

- GSH Experimental RC50 (Adverse outcome pathways).

As reported during the Inception Phase, we were not able to identify any new databases for the Skin and Respiratory Sensitisation endpoint (e.g. generated with novel in vitro assays described in this section) in addition to data already available in the OECD Toolbox v. 3.0.

We will develop vocabulary/ontology for those new assays despite the absence of actual database which may be further considered in the future once date become available. This will be like a separate ontology class with specific annotation to enable distinction from the actual ontology of the Toolbox databases and will contain names of assays and other available information on the assay as such.

4.2 WP3 Skin and Respiratory Sensitisation: Deliverables 2.1 "Definition of classes and hierarchical relationships in the ontology structure". Detailed work description.

It is important to notice that the WP3 Ontology covers the Skin Sensitisation Endpoint, since the OECD TGs, most of OECD Toolbox sensitisation datasets and all alternative experimental assays are for the skin sensitisation only. The respiratory sensitisation is mentioned only for the class "HT 66-2 Respiratory sensitisation" and within the headers for the "in chemico" databases where it can be relevant.

During the WP3 D4.1 implementation we have indentified and defined classes and hierarchical relationships for the Skin and Respiratory Sensitisation ontology.

The present ontology has to respect regulatory needs, and was tailored on existing constraints, i.e.,: a) the OECD HTs; and b) the experimental QSAR Toolbox databases. The goal of the project is to support and improve data integration and mapping in Toolbox, which is very domain-specific task, with most terms/concepts already having an official terminology (used e.g., in the OECD HTs).

To guarantee the compatibility of ontology with the OECD QSAR Toolbox, the ontology was separated into two main super-classes: the first one is dedicated to HTs: 66-1 Skin sensitisation (including draft version) and 66-2 Respiratory sensitisation, while the second covers the experimental datasets and assays terminology. Using this approach, it is possible to add smoothly any experimental sensitisation dataset to the ontology.

The most important standard that has been preserved during the ontology implementation is the OECD Harmonized Templates (OECD HTs) [102]. We have conserved all documentation and the structure of the OECD Harmonised Templates 66-1/66-2 and relative picklists [104] (Figure 42).

The conversion of the HTs into the OWL format was performed semi-automatically, with the Protégé Excel Import Plug-in. The OECD field number and other information contained in the HTs documentation was maintained in order to facilitate the ontology maintenance in case of HTs updates.

When possible we have imported the SC1 Ontologies parts mostly for the overlapping administrative terms of the HT. The rest of work has been done from scratch.

The second part of the ontology "Ontology for experimental assays" covers

- the skin sensitisation databases from the OECD QSAR Toolbox software v.3 (see section 1 Background),

-new alternative methods in vivo and in vitro despite the absence for some of them of actual databases,

-OECD Test Guidelines for skin sensitisation.

It was agreed to delete the AOP databases line from the owl file, and to create 3 different headers (Figure 43):

1. In vivo (skin sensitisation, and skin sensitisation ECETOC)
2. In vitro (Dendritic cell activity, and Keratinocyte gene expression databases)
3. In chemico (chemical reactivity and GSH reactivity databases)

This structure is also in line with the structure of these databases within the QSAR Toolbox.

Based on a careful preliminary survey we did not obtain any information about the existence of new systematic databases not included into the Toolbox, e.g. data generated with novel in vivo assays [113]. We have included the short description for these assays with vocabularies under the respective TGs classes in separate ontology class "alternative test methods" (Figure 44). For alternative assays in vitro and in chemico, which are still under development, the literature links have been collected for better definition of these test. In such a way the

ontology concept will be kept flexible and it will be possible to extend the ontology to cover new datasets when and if available in the next future.

The subclass “OECD Test Guidelines” covers the following Test Guidelines for skin sensitisation (Figure 45):

6. OECD TG 406: Skin Sensitisation: Skin Sensitisation;
7. OECD TG 429: Skin Sensitisation: Local Lymph Node Assay;
8. OECD TG 442a: Skin Sensitization: Local Lymph Node Assay: DA;
9. OECD TG 442b: Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA:
10. We will also take in consideration the Proposal for a template, and guidance on developing and assessing the completeness of AOPs
11.

The general structure of the ontology is shown in Figure 46.

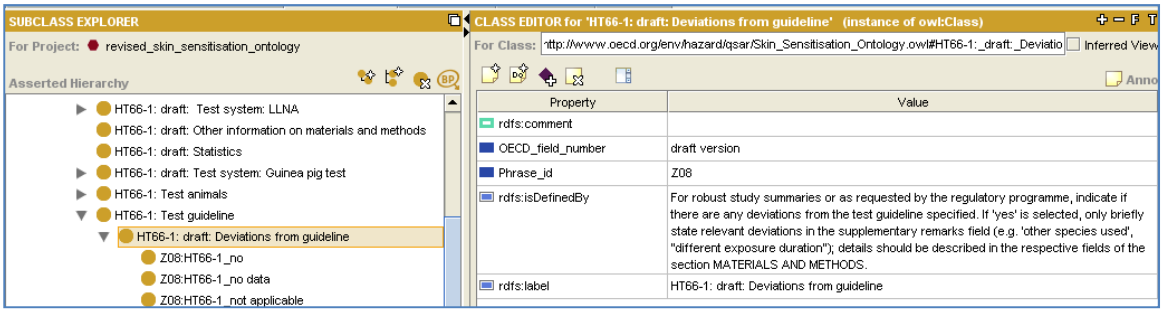


Figure 42. WP3.Protégé Screenshot: the HT’s fragment in OWL format.

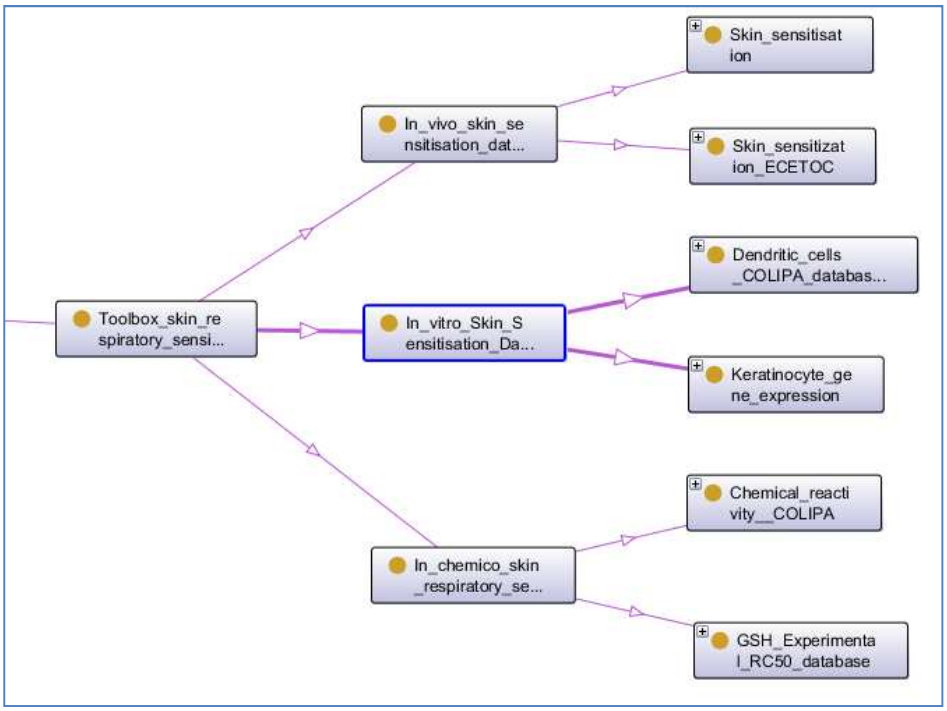


Figure 43. WP3.Separate sub-class describing the skin sensitisation databases.

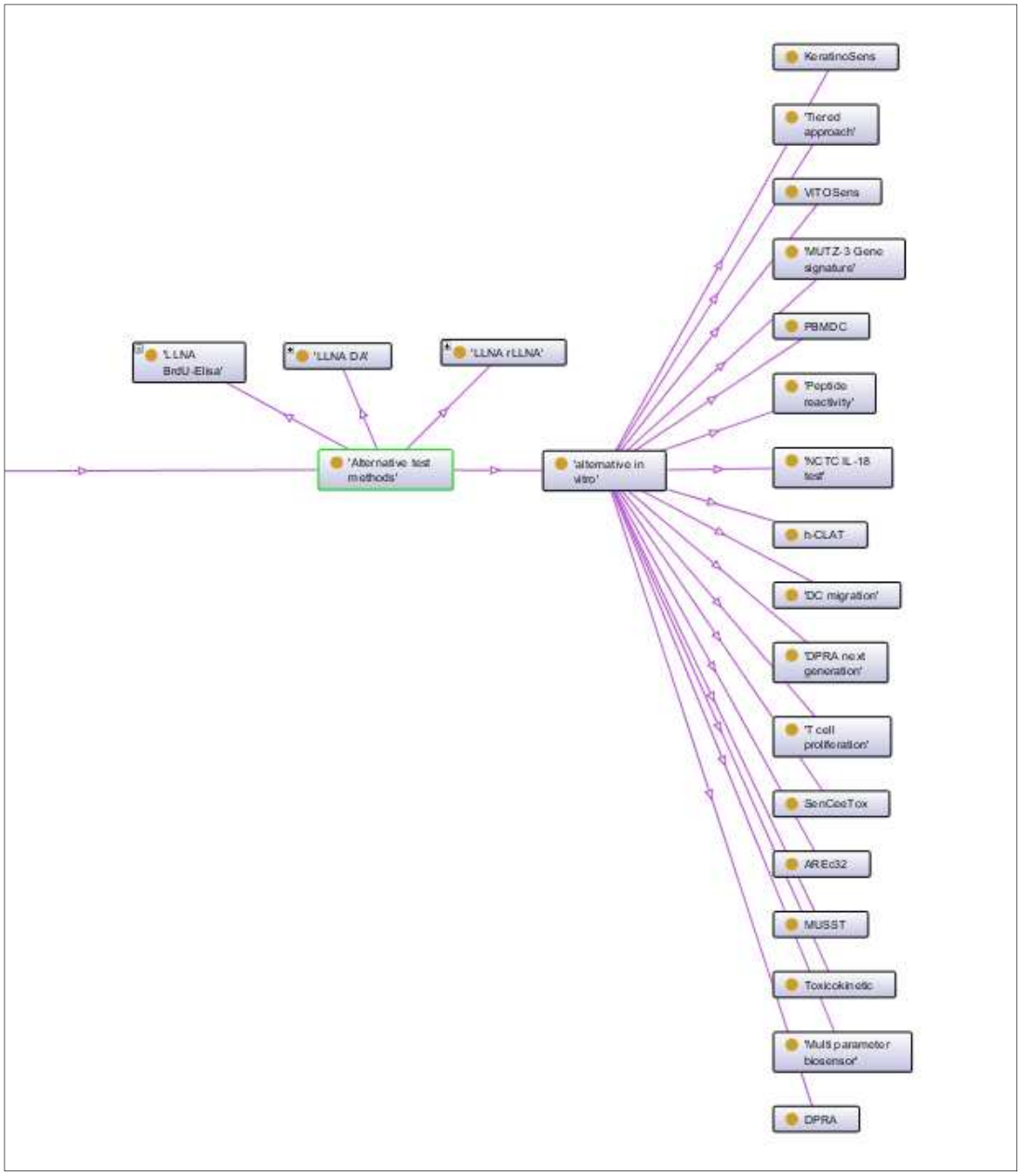


Figure 44. WP3.Separate sub-class describing alternative assays.

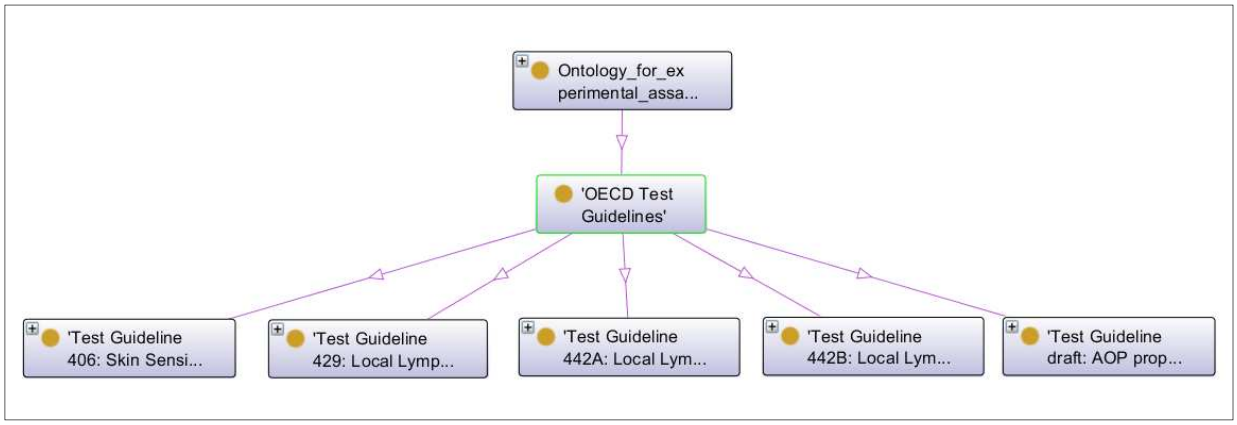


Figure 45. WP3.Separate sub-class describing OECD Test Guidelines.

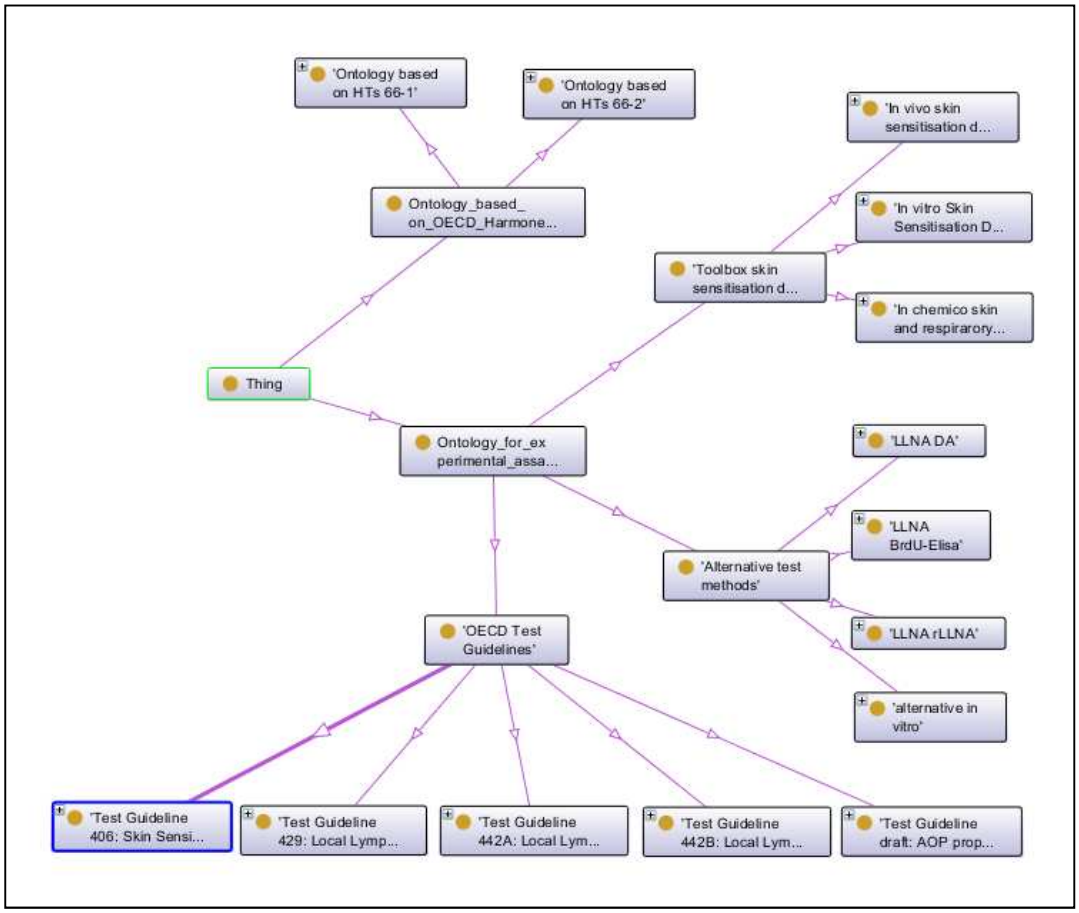


Figure 46. WP3.General structure of the Skin and Respiratory Sensitisation ontology.

4.3 WP3 Skin and Respiratory Sensitisation: Deliverables 4.2 "Compilation of terms related to the endpoint". Detailed work description.

In addition to the terms collected from the QSAR Toolbox datasets additional terms have been collected from different sources including:

- OECD Harmonised Template 66-1 and 66-2 (see an example on the Figure 47). Since some important information (OECD fields numbers, picklists numbers, etc) is missing in the draft version, it has been decided to add the prefix "draft" to all term, extracted from the draft HT. This will facilitate the update of the draft terms to the final ones;

-OECD HT #66-1 and 66-2 free test fields extracted from the HT text documentation (Figure 48);

-OECD Test Guidelines, mentioned in the previous section. Additional terms have been found in the Test Guidelines mentioned below and translated into the OWL format (Figure 49);

-Additional sources describing the alternative in vivo assays [113] (Figure 50) and alternative in vitro assays described in the Inception Phase Report.
Different prefixes have been used for the class label in order to facilitate the further ontology maintenance and possible update:

"Draft:" as explained above for terms, extracted from the draft version of HT;

prefix as a code of the HT picklist, for example, "T02-10:HT66-2_hamster", where "T02-10" is the picklist code for the class "Species";

prefix for terms extracted from different OECD Test Guidelines, for example, "Test Guideline 429: Local Lymph Node Assay: LLNA", where "TG429:" indicates the TG;

prefix for terms extracted from the OECD QSAR Toolbox databases, for example, "OECD QSAR Toolbox v3 dataset: Reference source".

For some terms the explicit links to the relevant external ontologies have been provided, e.g.

Phenotypic Quality Ontology http://purl.bioontology.org/ontology/PATO;

The NCBITaxon ontology (a taxonomic classification of living organisms and associated artifacts) http://purl.bioontology.org/ontology/NCBITAXON;

The EFO (Experimental factors ontology) http://www.ebi.ac.uk/efo/EFO_0000433;

Units of Measurement Ontology http://bioportal.bioontology.org/ontologies/UO.

Overall about 1300 terms have been implemented in the skin sensitisation ontology.

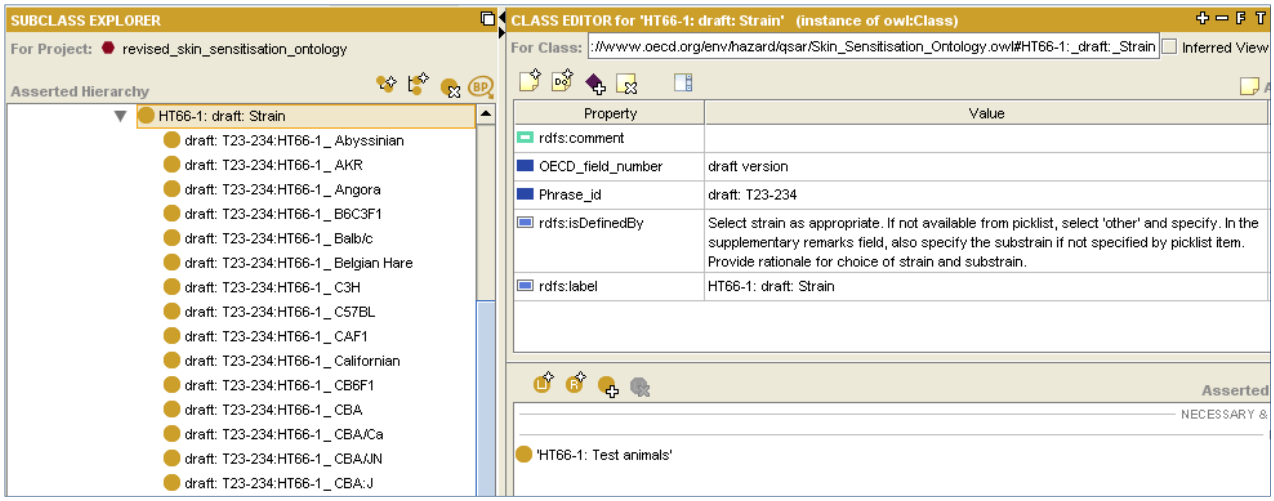


Figure 47. WP3.Example of translation of the draft HT version into the OWL format.

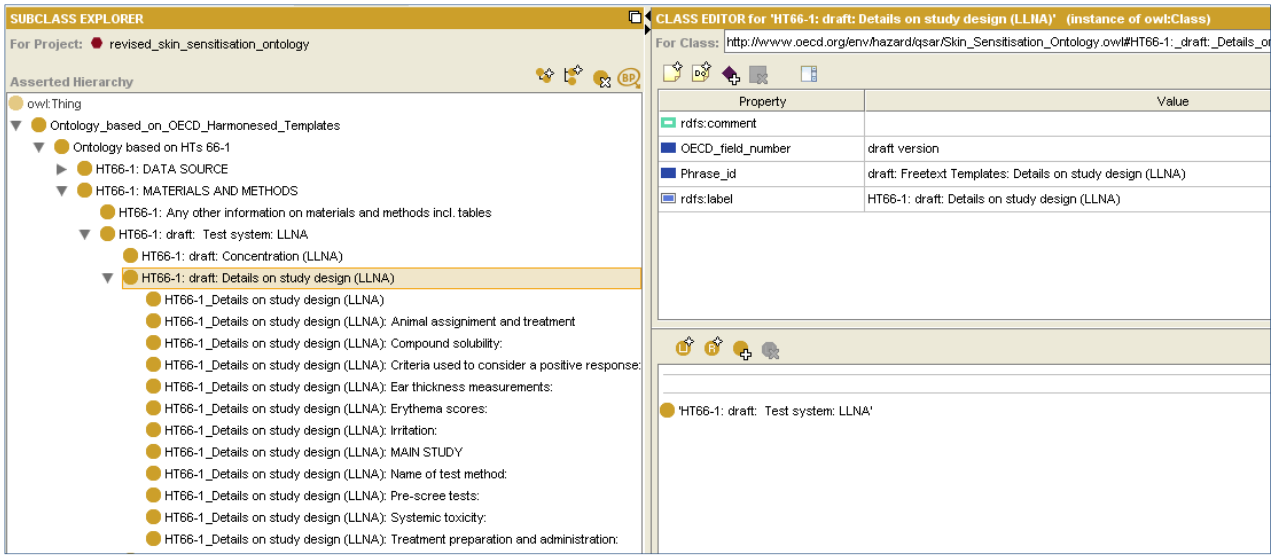


Figure 48. WP3.Example of translation of the free text definition in the OWL classes.

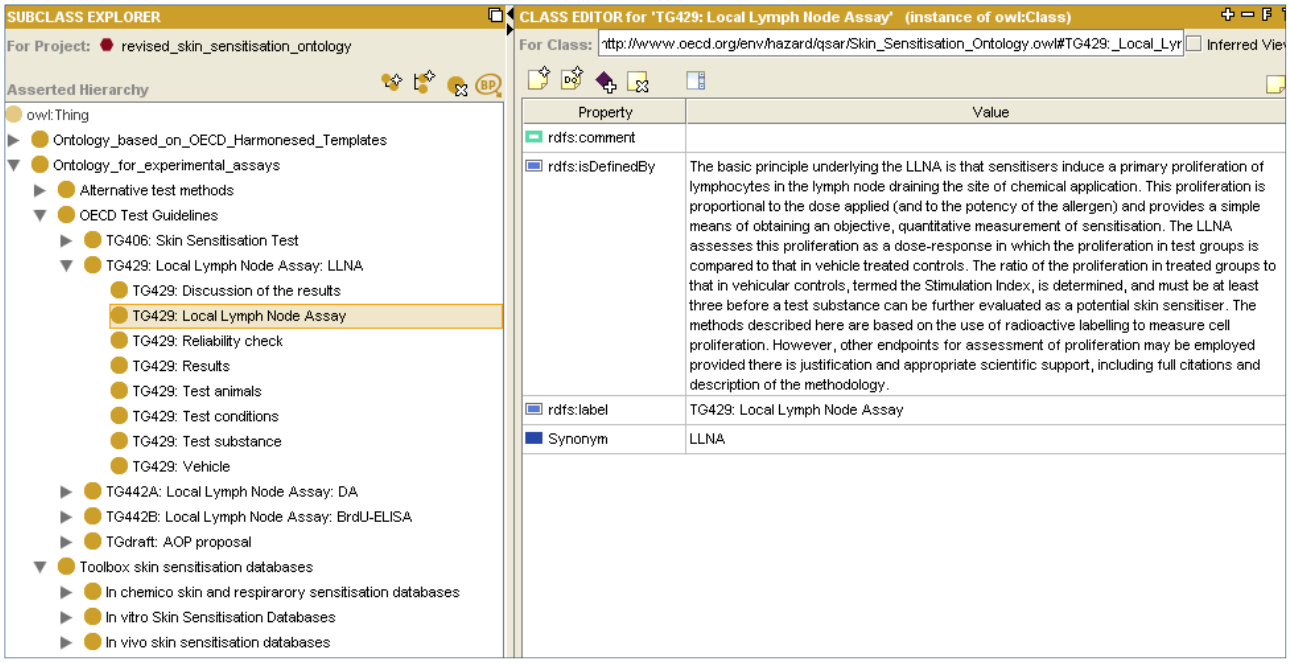
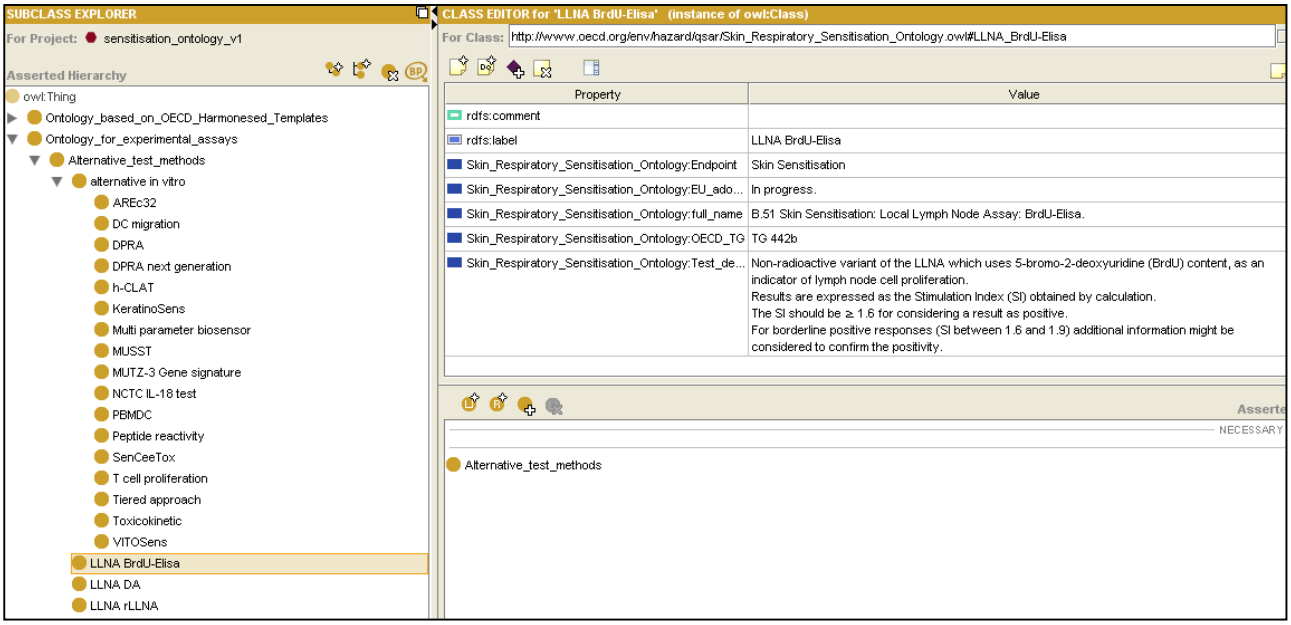
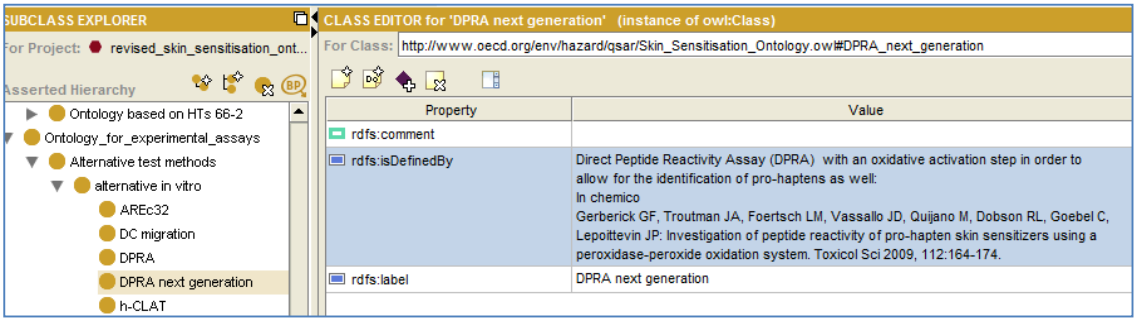


Figure 49. WP3.Example of the ontological description of the OECD Test Guidelines.



a



b

Figure 50. WP3.Examples of definition of the alternative assays: A-assay with OECD TG available, B-assay under evaluation and development,

4.4 WP3 Skin and Respiratory Sensitisation: Deliverables 4.3 "Definition of synonyms and homonyms". Detailed work description.

Synonyms and homonyms collection is needed for understanding how the same terms are defined and annotated in different sources, e.g. in the different datasets, the OECD HTs, the external biomedical ontologies. The OHTs terms, if available, are used as standard names.

The synonyms definition for single terms has been performed by introducing the OWL annotation property: "Synonym" using the multiple annotations of type Synonym, containing just one synonym string.

An example of such synonyms definition are shown on Figure 51. In the Toolbox the sensitising potency for the skin sensitisation dabase is described using different not standardized terms:

"Strong sensitizer" is also defined as "Strongly positive", "Strongly sensitising", "Category A";

"Non sensitizer" is also defined as "Not sensitising", "Category C", "Negative";

"Weak sensitizer" is also defined as "Category B";

"Moderate sensitizer" is also defined as "Moderately sensitising"

Synonyms have been added both manually and automatically when imported from the external resource using the Protégé Bioportal Import plug-in.

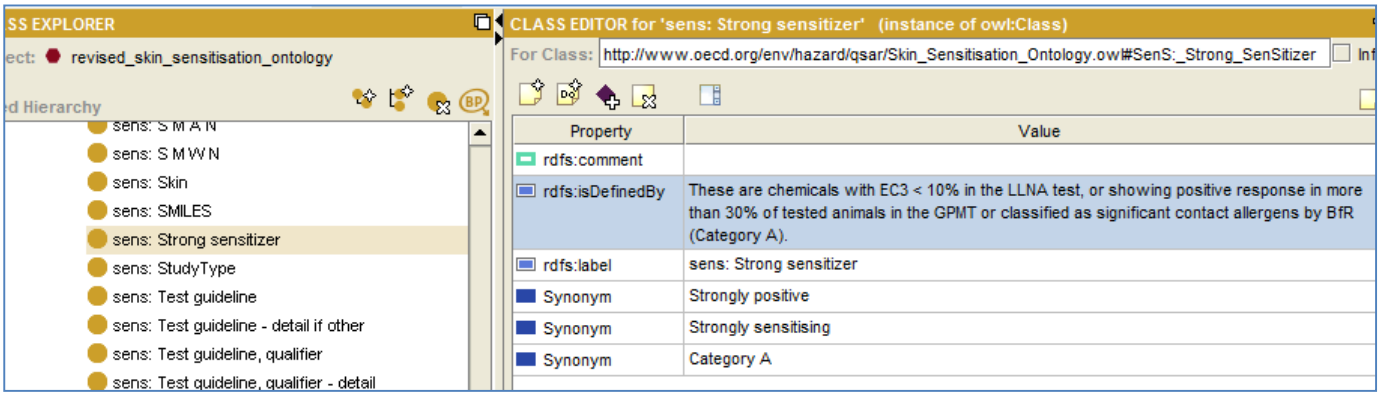


Figure 51. WP3.Examples of synonym definition using the objet property "Synonym".

4.5 WP3 Skin and Respiratory Sensitisation: Deliverables 4.4 Establishment of relationships, interactions and hierarchies between classes, objects and numerical properties for each term and rules when existing (internal rules and restriction rules).

In OWL language the relationships between ontological classes, internal and restriction rules could be introduced using the properties. The OWL language has three properties' types: object, annotation and datatype.

Object properties define the relationships between two classes and they are distinct from annotation and datatype properties. The role of annotation properties is to provide additional information (definitions, comments, etc) about ontology entities. Annotation properties do not participate in structural inferencing or reasoning. The role of datatype properties is to define the allowable data types and values for data values related to classes.

The Figure 52 shows the full list of object properties created during the Skin and Respiratory Sensitisation ontology development. For selected property "has_OECD_template_66_mapping" the textual definition (IsDefinedBy) as well as the corresponding domain and range are also shown (Figure 52).

The textual definitions for each property have been add to the OWL using the inbuilt property "IsDefineBy". Ranges and domains have been defined in OWL for all object properties.

For example, the property "is_covered_by_TG" and the relative inverse property "is_TG_for_method" permits the stabilization of the relationship between in vitro test methods and the corresponding OECD test guidelines. At the same time the property "has_OECD_template_66_mapping" permits the mapping of ontology terms (e.g. OECD TG) to the OECD HT 66 based ontology (Figure 53).

The terms of the Skin Sensitisation datasets have been mapped both to the OECD HT 65 based ontology and, when possible, to the corresponding OECD TGs terms using the property "has_TG_mapping" (Figure 54).

The numeric properties are simpler and have been used much less than the object properties, e.g. the property "has_data_type" (integer) has been used to the Test Duration definition in the Skin Sensitisation database (Figure 55).

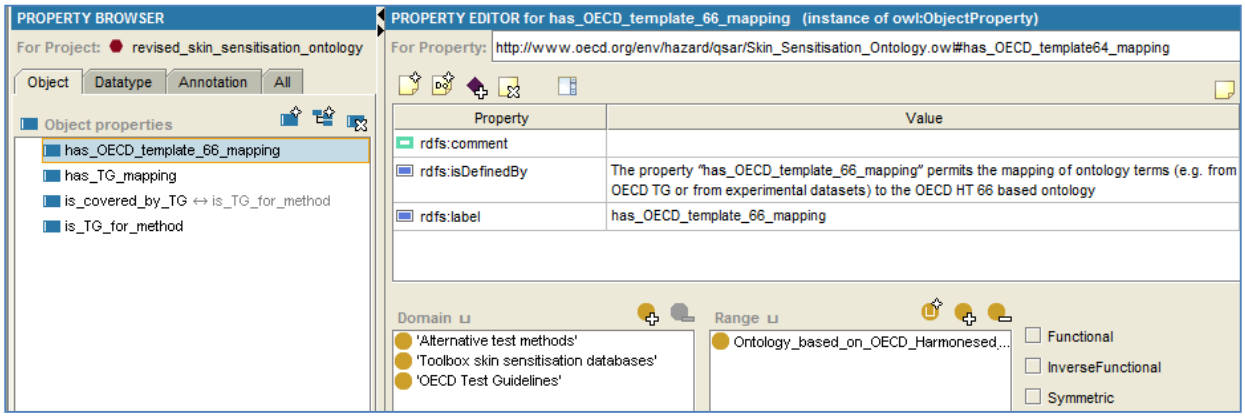


Figure 52. WP3.Protégé Screenshot: object properties created during the skin and respiratory sensitisation ontology development.

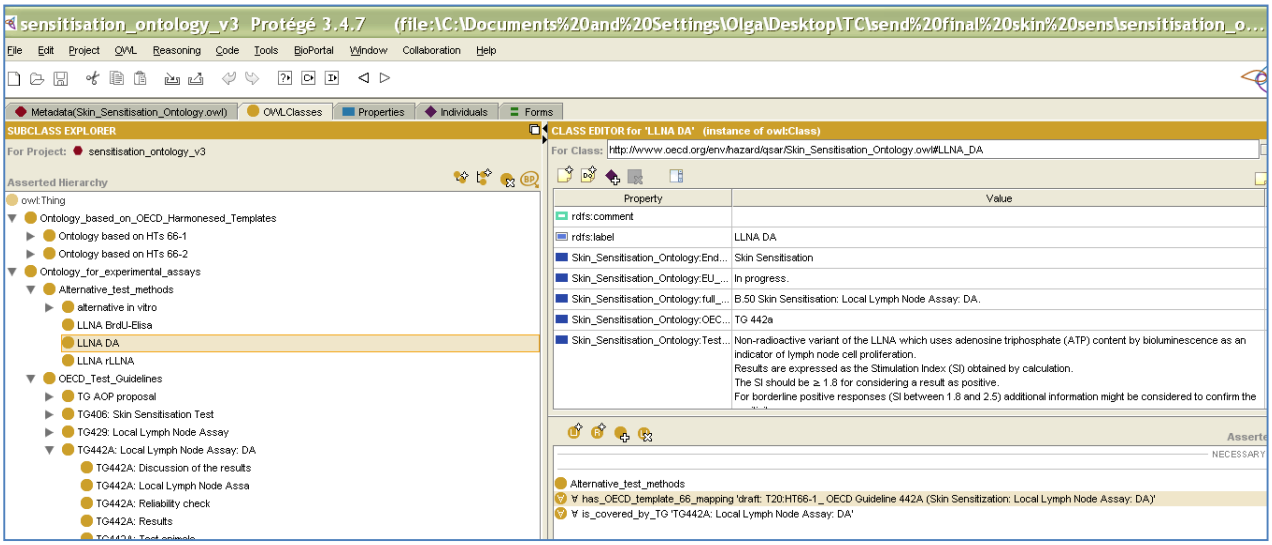


Figure 53. WP3.Protégé Screenshot: example of test guideline terms related to the corresponding test methods and mapped to the HT 66 based ontology.

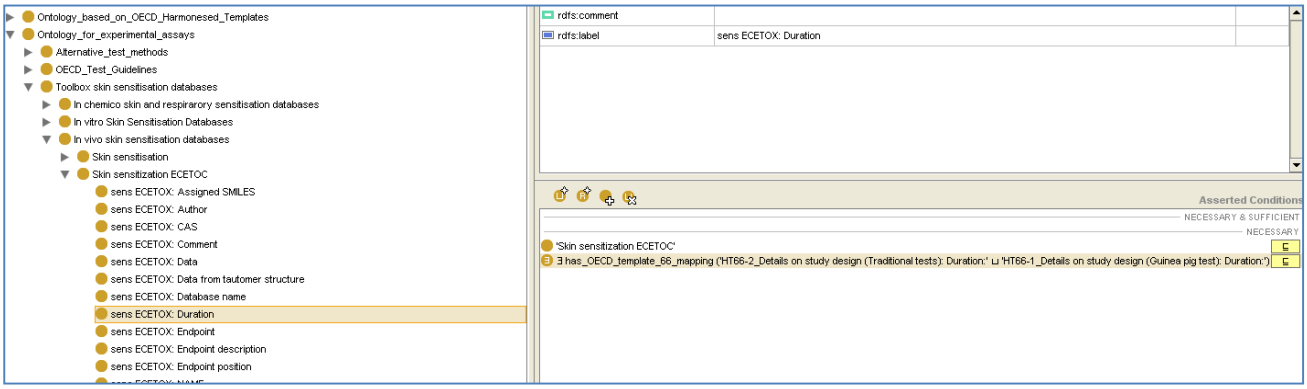


Figure 54. WP3.Protégé Screenshot: example of mapping of experimental dataset's term to the OECD HT and OECD TG ontology.

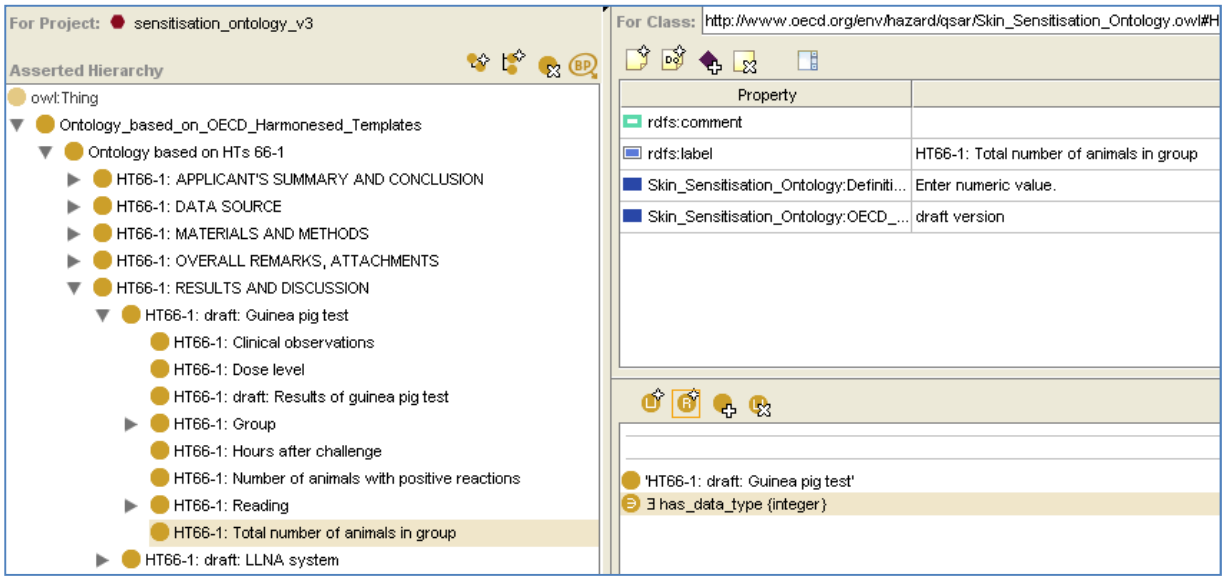


Figure 55. WP3.Protégé Screenshot: example of numeric property use.

The ontology metadata have been added using the Inbuilt Protégé annotation properties: “ontologyName”, “created_by” and “ontology version” (Figure 56)

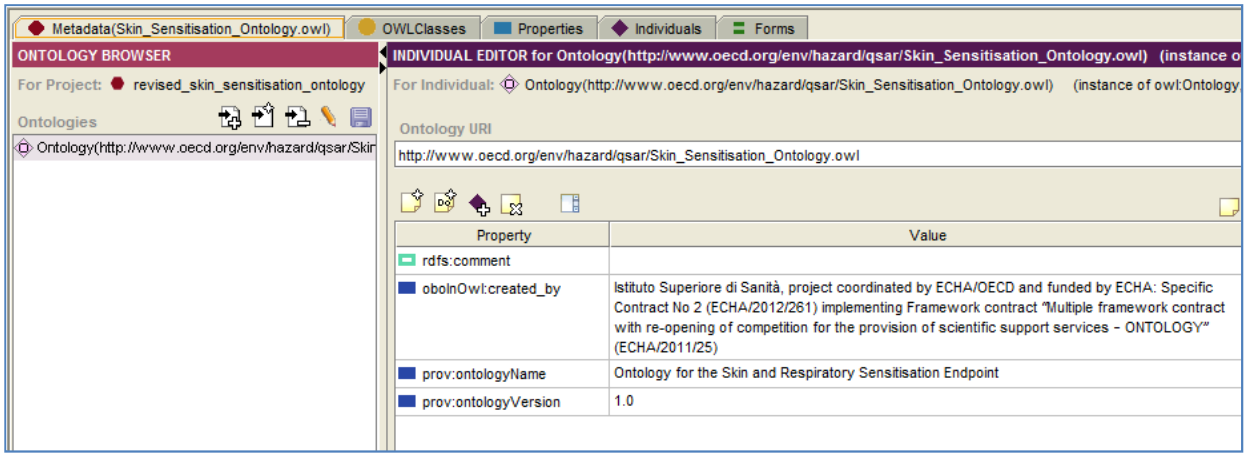


Figure 56. WP3.Protégé Screenshot: ontology metadata.

The full list of the properties (object, annotation and datatype) and textual explanation for each property are shown in the Table 9.

Property	Type of Property	Definition
has_OECD_template_66_mapping	Object	The property "has_OECD_template_66_mapping" permits the mapping of ontology terms (e.g. from OECD TG or from experimental datasets) to the OECD HT 66 based ontology
has_TG_mapping	Object	The property "has_TG_mapping" permits the mapping of ontology terms (e.g. from experimental datasets) to the OECD Test Guidelines terms.
is_covered_by_TG	Object	The property "is_covered_by_TG" and the relative inverse property "is_TG_for_method" permits the stabilization of the relationship between experimental test methods and the corresponding OECD test guidelines.
is_TG_for_method	Object	The property "is_covered_by_TG" and the relative inverse property "is_TG_for_method" permits the stabilization of the relationship between experimental test methods and the corresponding OECD test guidelines.
IsDefinedBy	Annotation	Inbuilt Protégé property used for definition of classes
Comment	Annotation	Inbuilt Protégé property used for definition of classes
Endpoint	Annotation	Indicates the toxicological endpoint for which the method is used
EU_adoption_status	Annotation	Status of OECD adoption for the method
EU_method	Annotation	Indicates the EU protocol for the method described
full_name	Annotation	Full method's name
OECD_field_number	Annotation	Field number imported from the OECD Harmonised template
OECD_TG	Annotation	OECD_TG
Phrase_group_id	Annotation	Number of the corresponding OECD picklist
Phrase_id	Annotation	OECD numeric code for the corresponding picklist
Synonym	Annotation	Synonyms collected for the term
Test_description	Annotation	Brief test description
Usage	Annotation	Indicates the toxicological endpoint for which the method is used
created_by	Annotation	Defines ontology's metadata, inbuilt Protégé property
creation_date	Annotation	Defines ontology's metadata, inbuilt Protégé property
ontologyName	Annotation	Defines ontology's metadata, inbuilt Protégé property
ontologyVersion	Annotation	Defines ontology's metadata, inbuilt Protégé property
has_datatype	Datatype	The property allows to specify allowed data types for some numeric terms (number of animals, duration, etc).

Table 9. WP3. Complete list of properties used in the OWL file

4.6 Conclusions

The Final Report and the Final version of the Ontology (OWL file in Protégé software) have been prepared for WP3 Skin and Respiratory Sensitisation after implementation of Deliverables 4.1-4.5.

The first main class of Skin Sensitisation ontology is based on OECD HTs: 66-1 (including draft version) and 66-2. The OECD field number and other information contained in the HTs documentation have been completely translated into the OWL format.

The second main class covers QSAR Toolbox Skin and Respiratory (only for in chemico data) Sensitisation databases' vocabulary, alternative in vivo and in vitro assays and relative test guidelines terminology (Deliverable 4.1).

The terms collection for the databases present in the Toolbox, OECD Test Guidelines, HT picklists and free text templates, for the alternative tests and synonyms definition have been performed during implementation of Derliverables 4.2 and 4.3. The ontology has been completed introducing relationships between classes using the OWL properties and restriction rules (Deliverable 4.4).

The Final Report (Deliverable 4.5) has been prepared.

The full list of the properties (object, annotation and datatype) and textual explanation for each property are shown in the Table 9.

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