

**ICCS**

**INTERNATIONAL  
COLLABORATION ON  
COSMETICS SAFETY**

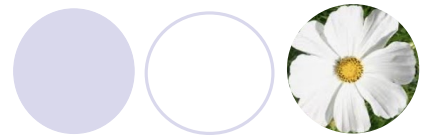
## THRESHOLD OF TOXICOLOGICAL CONCERN APPROACH FOR BOTANICAL SUBSTANCES

This document is prepared for the purpose of providing a pre-read for the workshop. The description of materials and analysis are still preliminary and results may change with further revisions. Hence we ask you not to distribute this document.

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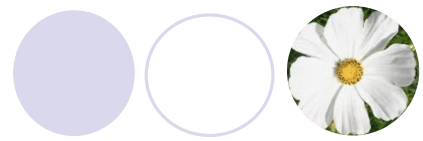
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## 1.0 Introduction

The Threshold of Toxicological Concern (TTC) concept is a risk assessment tool used in the evaluation of low-level exposure to substances where toxicity data may be limited or unavailable (WHO 2015, EFSA 2019; SCCS 2012/2023). TTC involves establishing a threshold below which the risk of adverse effects is considered negligible. Use of this method has been applied to direct/indirect food additives, flavorings & fragrances, and cosmetic ingredients and impurities. The goal of this project is to establish methods to extend TTC approaches for the safety assessment of botanical mixtures which can be considered a highly conservative approach to exposure-based waiving. Furthermore, another goal is to be able to address point of departure from systemic/target organ endpoints as well as cases driven by reproductive and developmental findings. This project will establish practical strategies to assist safety assessment of botanical mixtures by evaluating current TTC approaches and developing a relevant working framework.

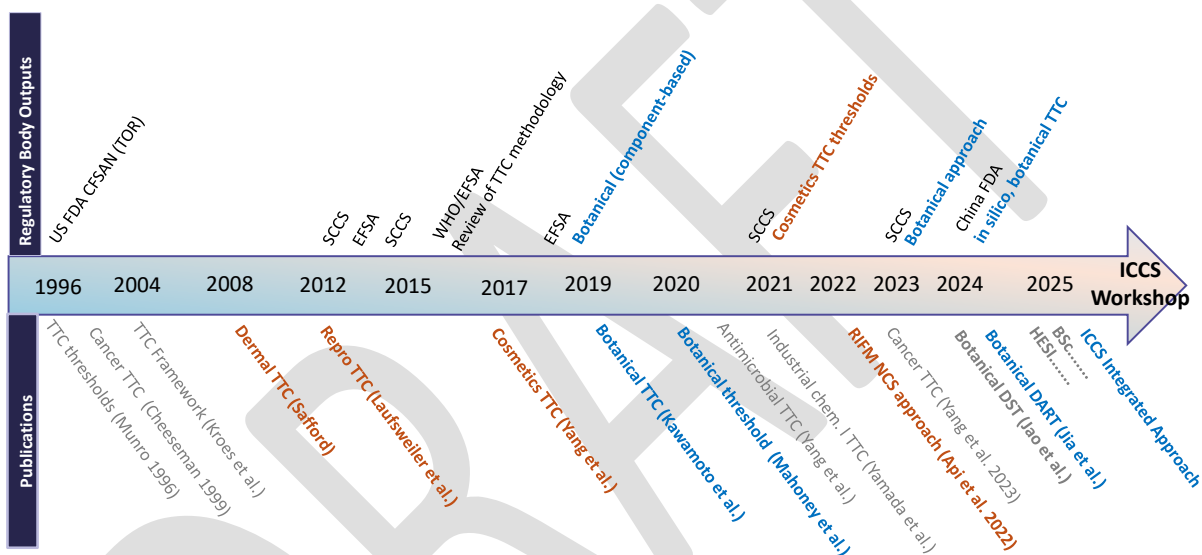
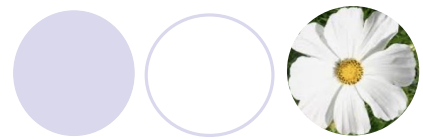


Figure 1 Evolution of TTC Approaches Designed for Specific Substance Groups

As shown in Figure 1, three data sets have been described in publications related to botanical TTC, i.e., repeated-dose toxicity (Kawamoto et al. 2019), genetic toxicity potential (Mahoney et al. 2020), and developmental and reproductive toxicity (Jia et al. 2024). Repeated toxicity data for 213 botanical extracts were analyzed to propose 663 mg/day as safe exposure while confirming that 90 mg/day seemed adequately safe. For potentially genotoxic substances in botanicals, meta-analyses on 183 botanical constituents resulted in a proposed threshold of 10 mg/day (dry wt) of plant material on a dry weight-basis per person per day. For DART TTC, interim results were recently reported (Jia et al. 2024) based on data from 67 phytochemicals and 108 botanical mixtures for potential DART (developmental and reproductive toxicity) effects and their possible reference doses, confirming that 90 mg/day seemed protective for botanical mixtures. Although these approaches and datasets were developed independently without a common base, they provided valuable resources and a good starting point for this project.

To this end, this project set out to build a new database designed to integrate repeated dose, developmental/reproductive, and genetic toxicity endpoints to represent the domains of targeted



substances, with emphasis on botanicals and phytochemicals. Point of Departures (PODs) are integrated from systemic/target organ, maternal/parental, reproductive, and developmental categories. Botanical substances are characterized to represent their specifications and connected to the component library of phytochemicals. In particular, we developed one threshold with the aim of being protective of three POD categories. It is also one of our ultimate goals to relate the botanicals in the database to phytochemical space and establish understanding of domain of applicability. Based on the integrated threshold, we therefore propose a decision framework to run the Botanical TTC Tree with the assistance from the currently available 10 µg of dry plant materials per person per day threshold for botanicals having genotoxic potential. When the tested plant material is not excluded (due to being outside the botanical domain of applicability) and not considered to have genotoxicity potential, our new threshold would ensure the safety of the botanical entity with low appreciable risk.

## 2.0 COSMOS Botanical Database

The new botanical database has three domains:

- 1) botanicals
- 2) chemical components (i.e., phytochemicals) found in the plant materials
- 3) toxicity studies

High level relationships of these three domains in the database are depicted in Figure 2. For toxicity content, two existing datasets, namely a TTC dataset for RDT (repeated-dose toxicity) (Kawamoto 2017) and a DART dataset (Jia et al. 2024), became the initial sources. The genetic toxicity domain (in vitro and in vivo) was also included to support the TTC approach.

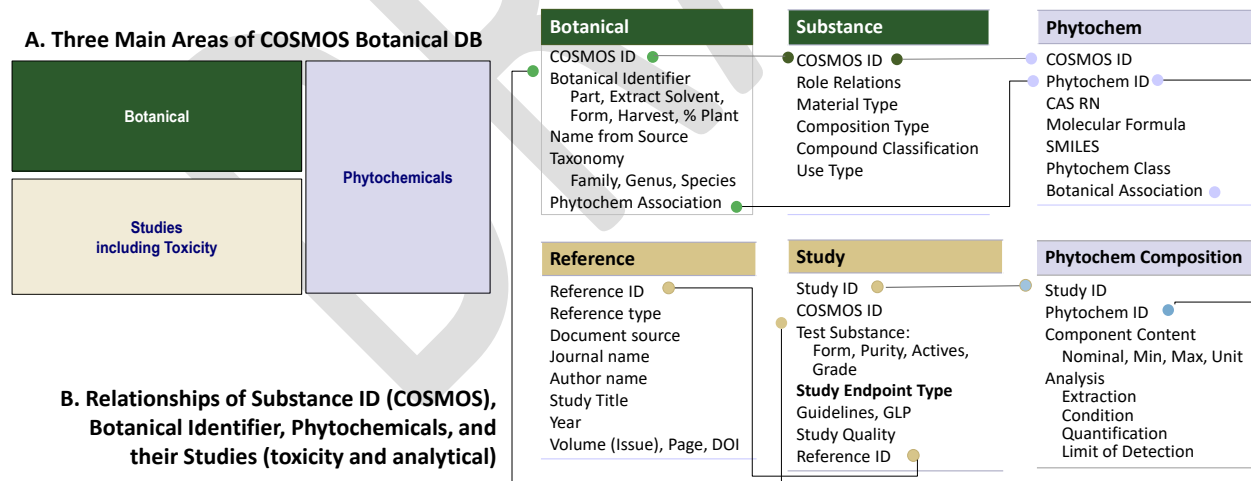
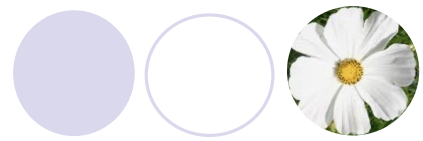


Figure 2. Domains and Relationships in COSMOS Botanical Database  
Study Endpoint Types includes Botanical Composition, Repeated Dose, Reproductive/ Developmental and Genetic Toxicity studies.



## 2.1 Botanical Substances

In designing a new botanical database to support TTC approach, we reviewed the existing resources from various sources, including the EFSA Compendium of Botanicals (EFSA 2025). EFSA’s database domain was focused on naturally occurring substances of possible concern for human health when present in food, covering a total of 863 unique scientific names from 131 botanical families with 535 genera. The goal of our project is to extend the botanical domain to include general substances such as cosmetics and dietary supplements beyond the food and flavors. The COSMOS Botanical database is currently comprised of 287 species (368 unique botanical substances) covering 94 families with 210 genera.

### 2.1.1 Botanical characterization

**Botanical Identifier.** Botanicals are characterized by their taxonomy (family, genus, species) along with the specifications including parts, extraction preparation method (solvent or extracting method), harvesting region, final extract form, and content of plant material in the extract. An example of “green tea extract” is given in Table 1 to explain the biological information captured in the database.

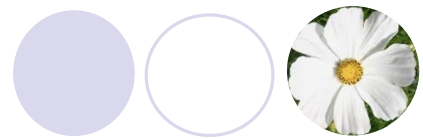
Table 1 Data Elements Describing a Botanical Entity

Characterization	Data Element Name	Example
<b>Botanical Identifier</b> (unique substance)	Test Substance Name (from Source)	Green Tea Extract
	Taxonomy: Family_Genus_Species	Theaceae <i>Camellia sinensis</i>
	Botanical Identifier	<b>CAMELIA SINENSIS_LEAF_STEAM/AQUEOUS_POWDER</b>
	Plant Parts	<b>LEAF</b>
	Extraction Solvent/Method	<b>STEAM/AQUEOUS</b>
	Harvesting Source	US COMMERCIAL
<b>Test Substance Specifications</b> (unique study)	Tested Form	<b>POWDER</b>
	% Purity: Plant Material in Final Extract	PLANT MATERIAL: ca. 90%
	% Actives	EGCG (48.4% wt)
	Grade	STANDARD: US PHARMACOPEIA   EXTRACTION YIELD: %UNSPECIFIED
<b>Substance Information</b>	Substance Material Type	BOTANICAL – NATURAL MIXTURE
	Substance Composition Type	SINGLE PLANT ENTITY
	Substance Use Type	nutraceuticals, dietary supplement, herbal medicine, flavor, beverage, cosmetics
	Associated Phytochemicals*	catechins (EGCG), theaflavins, caffeine, and L-theanine

\*Phytochemicals extracted from the botanicals are stored in the chemical structure-based phytochemicals table. (See section 2.2 & Table 3)

### 2.1.2 Botanical Substance Inclusion Criteria

This database includes only plant materials, hence bacterial fermentations or formulations employing animal products are excluded. Although we attempted to include mainly single-plant entities to make interpretation of NOAEL values and biological effects simpler, a good number of botanical substances have been reported in the literature for polyherbal formulations or multi-mixture preparations which contain numerous combinations of plant materials targeting particular biological outcomes, e.g., herbal medicines.



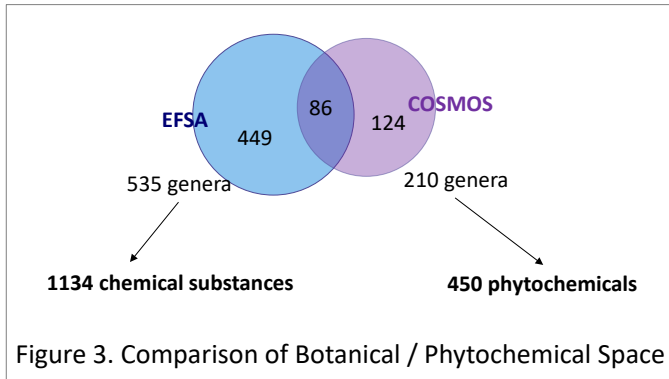
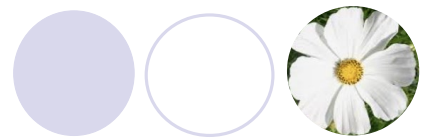
Due to this complexity of potency and biological activities, these are not included in TTC analysis. The simpler cases of binary or ternary compositions, ethnic medicinal mixture preparations, or their standardized commercial products were included. For example, Lomens-P0 is a 1:1 binary mixture of *Hordeum vulgare* and *Chrysanthemum zawadskii*. While taking the POD from the whole mixture for the database, the individual components were also added, whenever possible, i.e., the component or similar species within the same genus are considered, instead of calculating their potency contributions from the mixture. Table 2 illustrates several examples.

Table 2 Botanical Substances Representations in the COSMOS Botanical

Multi/Polyherbal Mixtures	Examples	Botanical DB	TTC Dataset
Mahwangyounpae-tang (MHYPT)	<b>A polyherbal formulation</b> consists of 12 plants including Massa Medicata Fermentata (3.9%); Semen Armeniacae Amarum (6.0%), etc.	Included	Excluded
ADP	<b>Formulated botanical mixture</b> consists of Anemarrhena rhizome + Phellodendron cortex) [1:1]	Included	Included (NOAEL as a whole mixture)
Components of ADP-	<ul style="list-style-type: none"> <li>• <i>Anemarrhena asphodeloides</i></li> <li>• <i>Phellodendron amurense</i></li> </ul>	<ul style="list-style-type: none"> <li>• Included</li> <li>• Included</li> </ul>	<ul style="list-style-type: none"> <li>• NOAEL included</li> <li>• NOAEL included</li> </ul>
AYAHUASCA	<b>Entheogenic herbal preparation.</b> Ayahuasca ( <i>Bandisteropsis caapi</i> + <i>Psychotria viridis</i> ) (2.1 – 4:1)	Included	Included (NOAEL as a whole mixture)
Components of AYAHUASCA	No data are found for the individual component of Ayahuasca.	No data	No data
INNOSLIM®	<b>Commercial product</b> consists of standardized composition of <i>A. Membranaceus</i> and <i>P. Notoginseng</i> (1:1)	Included	Included (NOAEL as a whole mixture)
Components of INNOSLIM	<ul style="list-style-type: none"> <li>• <i>Astragalus membranaceus</i></li> <li>• <i>Panax notoginseng</i></li> </ul>	<ul style="list-style-type: none"> <li>• Included</li> <li>• Included</li> </ul>	<ul style="list-style-type: none"> <li>• NOAEL included</li> <li>• NOAEL included</li> </ul>

In the preliminary version of the Database (dated 2025-10-30), of the 422 unique botanical substances, the collection of 54 polyherbal formulations, 17 simple mixture preparations, and 351 unique single-entity botanical substances are included. It is also possible to consider inclusion of EFSA food-related botanicals when clear toxicity information meeting our inclusion criteria is available.

## 2.2 Phytochemical Space in the COSMOS Botanical Database



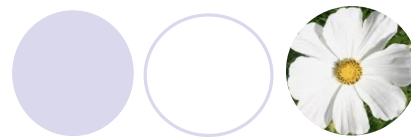
Plant materials in the COSMOS botanical database originated from 210 genera and 97 families. Fewer than half of the botanicals were from the same genera as the EFSA compendium, which may be explained by EFSA's focus on botanicals used in food. Figure 2 further indicates the chemical substances isolated/found in these plant materials – 1,134 chemical substances in EFSA and 450 phytochemicals in COSMOS. The chemical classes from the two sources are

actually quite different, which may be explained again by the low number of overlaps of botanical genera between the two and the fact that EFSA's inclusion was focused on the food-related substances of concern. We can confirm this observation through the Substance Use Type information defined in COSMOS database (Table 1).

Despite the diversity of chemical space within each species and genus, common phytochemical classes (structural features) can also be found across related species and genera. Both the EFSA Compendium and COSMOS Botanical databases can become the resource for establishing associations of the pairs of phytochemicals and botanicals. Eventually such a map will strengthen a domain of applicability for this approach. While the EFSA compendium lists the reported chemicals of possible concern, the COSMOS collection is directly related to phytochemicals extracted from 210 genera associated with dietary supplements, cosmetics, herbal medicine, or foods. It is one of the goals of this project that we provide a structure searchable database to search and visualize the associations between botanical species and phytochemicals. However, it is also important to note that neither COSMOS nor EFSA provides comprehensive coverage between plants and phytochemicals at this point.

Another resource for botanicals and phytochemicals is the LOTUS Natural Products database which was designed as a reference catalogue (LOTUS DB). It provides data for a large number of natural products including plant materials (over 27,953 botanical species or organisms) as well as the phytochemical structures in SMILES format (276,500). Whilst this is undoubtedly a great source, it should be noted that each molecule in the Lotus database is usually linked to only a single organism, the one in which it was first isolated or most definitively characterized. For example, a common chemical such as limonene (C=C(C)C1CC=C(C)CC1) is only once associated with *Carapichea ipecacuanha* in the Lotus database. In COSMOS Botanical, limonene is related to e.g., *Perilla frutescens* L., *Myristica fragrans* Houtt (Nutmeg oil), *Melaleuca alternifolia* (Tree oil), *Salvia officinalis* (Sage oil), and *Coriandrum sativum* (Coriander).

Based on scoping of the currently available resources, the structure-searchable phytochemical section of the COSMOS Botanical database will be a significant addition to botanical research. The structure of the database is illustrated in Figure 2B. Through this high-level diagram, we can see how botanical substances are linked to phytochemical components and then to the composition studies. Table 3 lists instances of this mapping through the database, taking examples of composition study results from *Camellia sinensis*\_Leaf\_Aqueous\_Powder (Green Tea Extract) and *Astragalus membranaceus*\_Leaf\_Aqueous\_Powder (huang qi or milk vetch). This association of botanical substances to phytochemicals can be further extended to map



from the species/genera level to chemical structural features (e.g., ToxPrint chemotypes). Eventually this genericized mapping will enable the definition of the applicability domain of the botanical TTC approach for untested plant materials. Briefly, Table 3 provides examples of phytochemicals containing the same/similar structural features that are associated within a given species/genus but not in others; these features can be used to guide the categorization of new botanicals and define applicability domain. At the same time, there are other features that are observed in similar chemicals across unrelated species/genus (e.g., features highlighted green in Table 3); these non-differentiating features are less useful for categorization. It's important to note that the features used to categorize new substances and define the applicability domain are important solely because of the structure information they capture, and whether or not they are associated with toxicity effects is irrelevant.

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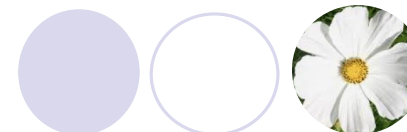
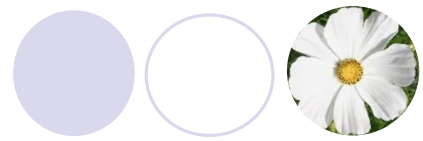


Table 3. Data Elements of Phytochemicals & Composition Analysis (Selected Records)

Botanical Substance Name	Phytochemical Name	Structure	Compound Classes & ToxPrint Names	Analysis Solvent	Analysis Quantitation	Analysis (%) Composition
<i>Camellia sinensis</i> _Leaf_Aqueous_Powder <i>Genera: camellia</i>	Epigallocatechin gallate (EGCG)		flavans, polyphenols hetero_[6]_O_pyran_generic ring:hetero_[6]_Z_generic bond:COH_alcohol_aromatic_phenol	EtOH:H2O	HPLC UV & MS	48.4
<i>Camellia sinensis</i> _Leaf_Aqueous_Powder <i>Genera: camellia</i>	Epicatechin gallate (ECG)		flavans, polyphenols hetero_[6]_O_pyran_generic ring:hetero_[6]_Z_generic bond:COH_alcohol_aromatic_phenol	EtOH:H2O	HPLC UV & MS	12.8
<i>Camellia sinensis</i> _Leaf_Aqueous_Powder <i>Genera: camellia</i>	Epigallo catechin (EGC)		flavans, polyphenols hetero_[6]_O_pyran_generic ring:hetero_[6]_Z_generic bond:COH_alcohol_aromatic_phenol	EtOH:H2O	HPLC UV & MS	2.26
<i>Camellia sinensis</i> _Leaf_Aqueous_Powder <i>Genera: camellia</i>	Epicatechin (EC)	<chem>OC1(C(OC2=C(C1)C(O)=CC(=C2)O))C3=CC(O)=C(C=C3)O</chem>	flavans, polyphenols hetero_[6]_O_pyran_generic ring:hetero_[6]_Z_generic bond:COH_alcohol_aromatic_phenol	EtOH:H2O	HPLC UV & MS	2.83
<i>Camellia sinensis</i> _Leaf_Aqueous_Powder <i>Genera: camellia</i>	Caffeine		purine alkaloids, methylxanthins group:nucleobase_xanthine_purine-2_6-dione ring:hetero_[6]_N_pyrimidine_2_4-dione ring:hetero_[6]_Z_generic	EtOH:H2O	HPLC UV & MS	4.9
<i>Astragalus membranaceus</i> _Leaf_Aqueous_Powder <i>Genera: Astragalus</i>	Quercetin		Hydroxyflavones, polyphenols ring:hetero_[6]_O_pyran_generic ring:hetero_[6]_Z_generic bond:COH_alcohol_aromatic_phenol bond:CC(=O)C_ketone_aromatic_aliphatic	TFA Hydrolysis	HPGPC / ELSD	0.321 mg/g
<i>Astragalus membranaceus</i> _Leaf_Aqueous_Powder <i>Genera: Astragalus</i>	Astragaloside IV		pentacyclic triterpenoid, <a href="#">cycloastragenol</a> group:carbohydrate_aldohexose;_aldopentose ring:polycycle_spiro_[2.5]octane ring:fused_steroid_generic_[5_6_6_6] ring:hetero_[6]_O_pyran_generic ring:hetero_[6]_Z_generic	TFA Hydrolysis	HPGPC / ELSD	0.161 mg/g



## 2.3 Toxicity Database

### 2.3.1 Toxicity Data Element Description

The toxicity database of the COSMOS Botanical TTC database follows the method described by previous COSMOS TTC publication (Yang et al. 2017). In general, there are four sections to describe toxicity data:

- 1) study design
- 2) study results
- 3) study quality and guidelines
- 4) reference

The following tables give a full description of the information captured for toxicity data relating to these four sections. Current data entry forms are provided with the OHT (OECD Harmonized Template) compatible pick list. Each data field is also controlled by a set of vocabularies reflecting the content inclusion criteria of the database. This data model and controlled vocabulary sets are critical for database analysis in this project.

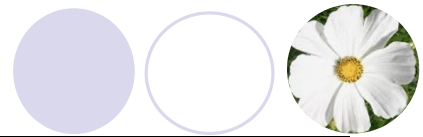
#### 2.3.1.1 Study Design

Date Element	Repeated Dose	DART
Study Type (Option controlled)	chronic, subchronic, sub-acute, short-term, combined chronic/carcinogenicity, combined repeated/repro-dev screening test, EOGRT	reproductive, repro-dev screening test, multigeneration, EOGRT, pre-/peri-natal developmental, etc.
Species with strain	rat, mouse, dog, monkey (species option controlled)	rat, mouse, rabbit (species option controlled)
Sex	Studies with both M/F preferred	Guidelines
Route of exposure	Oral routes (species option controlled)	Oral routes (species option controlled)
Duration	min. 28 days Dosing & recovery period information	clear dose duration information on pre-mating, mating, GD, PND, LD for both male and female
Doses	Clear doses and regimen Conversion information to mg/kg-bw/day (Unit option is controlled)	Clear doses and regimen Conversion information to mg/kg-bw/day (Unit option is controlled)

EOGRT: Extended One-Generation Reproductive Toxicity Study; GD: Gestation day; PND: Post-natal day; LD: Lactation day.

#### 2.3.1.2 Study Results

Date Element	Repeated Dose	DART
POD Category (Option controlled)	Systemic/Target organ	Reproductive/developmental: - Parental, Offspring, Reproductive Pre-natal developmental: - Maternal, Developmental
Original NOAEL	NOAEL or NOEL in mg/kg-bw/day	NOAEL or NOEL in mg/kg-bw/day
Original LOAEL	LOAEL or LOEL in mg/kg-bw/day	LOAEL or LOEL in mg/kg-bw/day
BMDL	When BMDL (mg/kg-bw/day) is calculated	When BMDL (mg/kg-bw/day) is calculated
GENERATION	ADULT: for repeated-dose toxicity	Reproductive/developmental:



(Option controlled)	- most cases adults are tested - Juvenile toxicities	- Parental, Offspring, Adult Pre-natal developmental: - Maternal, Embryofetal
CRITICAL SITES (Option controlled)	Site from Target organ findings at LO(A)EL (controlled vocabulary)	Sites from Target organs findings at LO(A)EL (controlled vocabulary)
CRITICAL EFFECTS (Option controlled)	Effects at LOAEL (controlled vocabulary)	Effects at LOAEL (controlled vocabulary)
STUDY GENERAL NOTES (Free text)	Comments to expand/qualify description of observed findings	Comments to expand/qualify description of observed findings
POD SOURCE (Option controlled)	The party who decided NOAEL values (Safety Assessment, Study Author, etc.)	The party who decided NOAEL values (Safety Assessment, Study Author, etc.)

### 2.3.1.3 Study Background Information

Date Element	Repeated Dose	DART
STUDY QUALITY	Klimisch score for reliability	Klimisch score for reliability
GUIDELINE	OECD TG, US EPA OPPTS, etc.	OECD TG, US EPA OPPTS, etc.
GLP	GLP compliance status	GLP compliance status

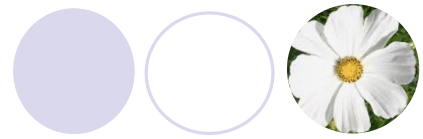
### 2.3.1.4 REFERENCE

Date Element	Studies
REFERENCE TYPE (Option controlled)	Regulatory Document, Database Report, Open Literature
DOCUMENT SOURCE	Document Provider Name
DOCUMENT SITE	Document Number, Technical Report Number
LITERATURE CITATION	Author
	Journal Name
	Volume (issue), Page
	Year
	Study Title
	DOI

### 2.3.2 Database Inclusion Criteria

Several criteria were considered in selecting the studies for both Repeated-Dose Toxicity (RDT) and Reproductive and/or Developmental Toxicity (DART) studies. Database inclusions are controlled during the data curation by designed fields and controlled vocabulary and again enforced during the general database quality control (QC):

- Repeated dose studies whose durations are shorter than 28 days are considered not long enough to provide reliable information on systemic/target organ toxicity.
- When systemic/target organ data are not available through repeated-dose toxicity studies, relevant information from reproductive studies can be employed.
- For developmental and teratology findings, if maternal effects were not examined, the studies were not deemed appropriate to be included in this database for the TTC approach. In other words, the developmental NOAEL values were considered only from studies where maternal toxicity data were available.



### 3.0 TTC Dataset & Analysis

#### 3.1 Transformation of Database to TTC Dataset

##### 3.1.1 POD Categories

Whilst the toxicity database may contain many studies for a specific endpoint for a given test substance, in the TTC approach it is customary to select the minimum POD values, if all other aspects are equivalent, to build a conservative dataset to best protect the public. In this project, since our goal is to evaluate the possibility of integrated TTC approach based on both systemic/target organ (repeated dose) and reproductive/developmental toxicities, our first task was to establish POD categories from various NOAEL values from relevant experiments.

Three POD categories are devised to combine NOAEL data from RDT and DART according to the process shown in Figure 4:

- Systemic/Target organ PODs are derived from NOAELs of repeated dose, maternal (prenatal developmental), and general parental toxicity (reproductive/developmental).
- Developmental PODs are derived from embryofetal (prenatal development) and offspring (repro/dev) developmental studies.
- Reproductive PODs are derived from fertility and reproductive performance findings from adult/parental generation in repro/dev studies.

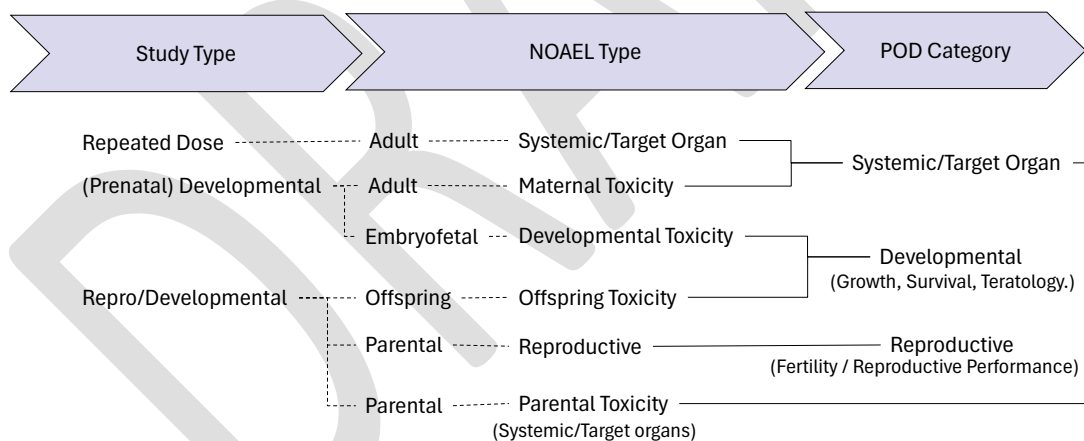


Figure 4. Schema for POD Categorization of Experimental Data

##### 3.1.2 POD Determination

For a given study type, a study that gives the minimum NOAEL value was selected to set the POD. The NOAEL values were adjusted for duration to standardize for long-term exposure. Typical adjustment factors are duration and LOAEL/NOAEL extrapolation. Tables 4.1 and 4.2 summarize various factors used in this study, consistent with other previously published TTC approaches.



Table 4.1. Conversion of NOAELs to POD Values

Transformations	RDT (systemic/target organ)	DART
Duration Adjustment Factor	<ul style="list-style-type: none"> <li>• 6X (28- ca. 40 day)</li> <li>• 3X (ca. 42 – 179 day)</li> <li>• 1X (&gt;=180 day)</li> </ul>	<ul style="list-style-type: none"> <li>• 6X (Male/Parental 28 day)*</li> <li>• 3X (M/F ca. 42 – 179 day)*</li> <li>• 3X (Multigen, 2 GEN)*</li> <li>• 1X (Multigen, 3 GEN)</li> <li>• 1X (Pregnant dams in development studies)</li> <li>• 1X (fetal or offspring developmental)</li> <li>• 1X (reproductive)</li> </ul>
LOAEL-To-NOAEL Extrapolation Factor	3X	3X
Adjustment Factors for BMDL	1X	1X
POD categories	Systemic/Target organ from RDT and OECD 421/422 or multigene studies	<ul style="list-style-type: none"> <li>• Reproductive (fertility or reproductive performance)</li> <li>• Developmental (embryofetal or offspring)</li> </ul>

\*Sensitivity analysis of the duration adjustment factors for the use of the systemic/target organ NOAELs from the reproductive-developmental toxicity studies confirmed that the factors do not affect the NOAEL distribution or the fifth percentiles.

Table 4.2. Data Elements for TTC Datasets: Results Section

Date Element	Repeated Dose	DART
POD Category	Systemic/Target organ	Reproductive Developmental
Adjustment Factor: Duration	Divide NOAEL by the factors listed in Table 4.1	Divide NOAEL by the factors listed in Table 4.1
Adjustment Factor: LOAEL-NOAEL Extrapolation	Divide LOAEL by 3	Divide LOAEL by 3
BMDL	1X when BMDL is calculated	1X when BMDL is calculated
logPOD	Log10(POD values)	Log10(POD values)

### 3.1.3 TTC Dataset

A TTC dataset was constructed by defining the inclusions from the two domains of the database, i.e., botanical substances and toxicity endpoints defined by POD categories. As discussed in section 2.1, a total of 368 botanical substances (350 single entities and 17 simply defined mixtures) were selected as the basis. For the POD categories, the RDT and Reproductive/Developmental studies have been integrated into one TTC dataset. Since the POD values range over many orders of magnitude and are not well-described by a normal (Gaussian) distribution, percentiles were estimated empirically from the raw quantiles, since these only depend on order and are not dependent on any assumed type of distribution. Results obtained for the four datasets are summarized in Table 5, where histograms are shown for  $\log_{10}(\text{POD})$  values to clearly see the wide range in values.

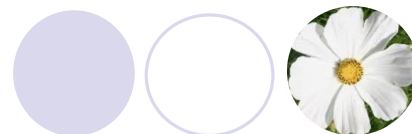
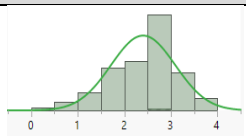
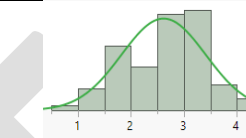
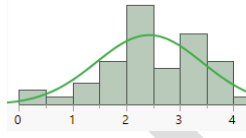
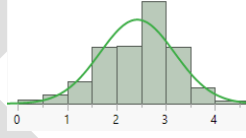


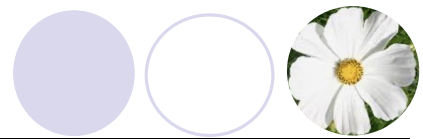
Table 5. Comparison of POD distributions for TTC Datasets

TTC Dataset	N (Size)	Dataset POD distribution (mg/kg/day)	Histogram (log POD)
RDT Systemic/target organ	253	100% 5074 50% 333.3 25% 100.0 5% 16.8 0% 2.3	
Developmental	105	100% 34500 50% 500 25% 100 5% 20.9 0% 3.5	
Reproductive	51	100% 34500 50% 266.7 25% 81.7 5% 2.0 0% 1.0	
Overall Minimum	368	100% 34500 50% 333.3 25% 80.8 5% 14.3 0% 1.0	

The overall minimum distribution was obtained by taking the minimum POD value from each botanical substance. Reproductive PODs are observed to be lower than RDT or Developmental. The lower end of the overall minimum distribution was influenced by a few substances known to be reproductive effectors. Table 6 lists some examples of the substances below fifth percentile.

Table 6 Examples from Lower 10% POD (mg/kg-bw/day) and High End of the Distribution

Scientific names*	RDT	Reproductive	Developmental	Phytochem association
<i>Ferula hermonis</i>		<b>3.0</b> [F] (fertility)		ferutinin
<i>Pradosia huberi</i>			<b>1.2</b> (embyo development)	proanthocyanidins/ tannins
A rusticana + allyl isothiocyanate	<b>3.1</b> (renal)	100.0	20.0	allyl isothiocyanate
<i>Ardisia squamulosa</i>		<b>3.3</b> Spermatogenesis		polyphenols (embeline) and phytosteroid
<i>Sanguinaria spp.</i>	<b>3.3</b> (body wt gain dec.;breathing)	100	20	benzophenanthridine alkaloid
<i>Ginkgo biloba</i>	100		<b>3.5</b> (growth reduction) under QC	Quercetin, kaempferol, Bilobalide, ginkgolide
<i>Tripterygium wilfordii</i>	<b>4.29</b> (liver, kidney, heart)	12.6 (spermatogenesis)		Triptolide
<i>Myristica fragrans oil</i>	<b>6.0</b> (liver)	59.3 (LOAEL 60-178, under QC)		Myristicin, phenylpropene (elemicin, saffron)
<i>Aristolochiae spp.</i>	<b>7.1</b> (renal)			Aristolochic Acid



<i>Pueraria mirifica</i>		<b>10</b> (ovary lesions, follicle growth, LH/FSH dec.)		Phytoestrogen (Miroestrol, Coumestrol, genistein, daidzein)
<i>Lawsonia inermis</i>			<b>10</b> (brain, skull, rib anomalies)	2-Hydroxy-1,4-naphthoquinone (henna)
<i>Melaleuca alternifolia</i>	<b>10</b> (male reproductive organs)	15 (fertility)	25 (at maternally toxic dose)	1,8-Cineole, alpha-terpinen-4-ol, alpha-Terpinene and gamma-Terpinene...
<i>Radix Ophiopogonis</i>		<b>29,000</b> (No adverse effects)		Antioxidant, saponins, flavonoids, polysaccharides, and steroidal compounds.
<i>Triticum Aestivum (non GM Wheat)</i>		<b>34,500</b> (No adverse effects)		Antioxidant, phenolic acids, flavonoids, saponins, and terpenoids.

A handful of botanical substances in the dataset resulted in low NOAELs from DART studies. The phytochemicals associated with these botanicals tend to be phytoestrogen, phytosteroids, polyphenols, and terpenoids. On the other hand, it is also the reproductive studies that populate the highest end of the data distribution. They are polysaccharides along with antioxidants. Further analysis of botanical-phytochemical associations as discussed in section 2.2 is planned to define the domain of applicability more clearly.

### 3.2 Fifth Percentile Determination

Cumulative distribution functions (CDFs) were constructed to confirm the shape of the data distribution curve, stability of the fifth percentile, and for comparison of various datasets, as shown in Figure 5.

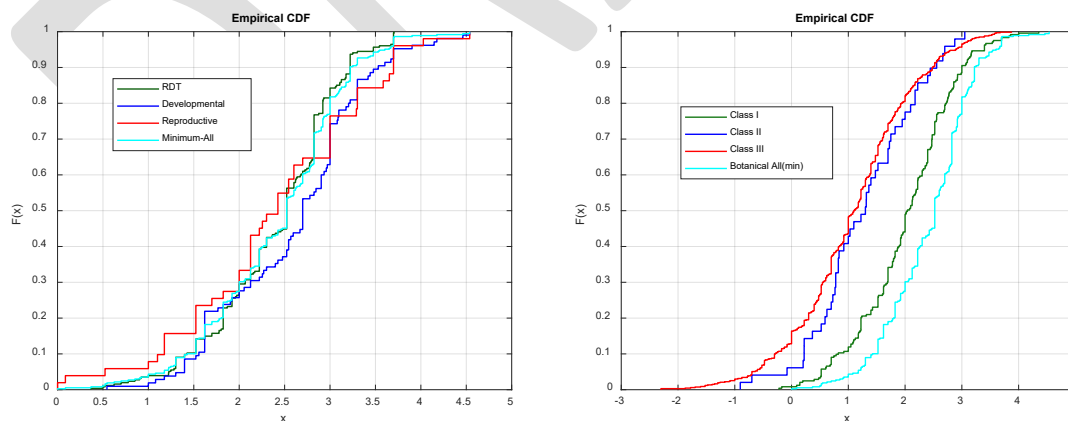
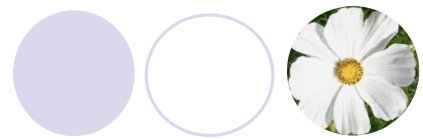


Figure 5 Cumulative Distribution Function (A: botanical dataset; B: botanical vs. chemical TTC)

As expected from the POD distribution shown Table 5, the four TTC datasets resulted in rather similar CDF shapes. Although the reproductive dataset gave a somewhat lower fifth percentile than that of RDT or Developmental, it roughly overlaps with the other two, considering the small dataset size. To integrate the TTC datasets, the minimum POD for each botanical substance was identified (of the three, RDT,



Reproductive, and Developmental), resulting in a sufficiently large (368) “Minimum-All” set was prepared. This curve results in a much smoother curve (without any curve fitting).

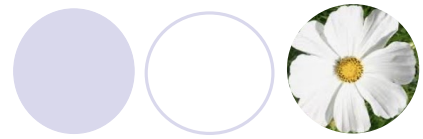
We then compared these botanical distributions to the cosmetics-rich chemical set (federated set). This federated set is defined as the federated combination of cosmetics TTC dataset (552) and Munro dataset (613) resulting in the 952 dataset (SCCS 2021; Yang et al. 2017). The CDF of the Minimum-All botanicals set gives a clearly higher fifth percentile than the Cramer Class I of the federated set. These two distributions can be statistically tested by non-parametric pair-wise Kolmogorov-Smirnov test (Conover 1999) to prove that botanicals are indeed less potent than the Cramer Class I of the federated chemical set. Two distributions are statistically different and botanicals give higher fifth percentile as well as the statistical moments (quartiles, median and mean) as summarized in Table 5. This analysis confirms that combination of the RDT and DART endpoints defined by POD categories to construct a minimum dataset for botanicals can indeed allow the integration of both RDT and DART approaches into a single botanical TTC approach.

Table 7. Preliminary Results from the Preliminary COSMOS Botanical TTC Dataset

Datasets	Fifth (mg/kg-bw/day)	Human (ug/person/day)*	N
Monro Class I	2.91	1800	137
Monro Class III	0.14	90	448
Federated Class I*	3.5	2760	243
Federated Class III*	0.22	138	671
RDT botanical	16.7	To be finalized	254
Developmental botanical	20.9	To be finalized	105
Reproductive botanical	2.0	To be finalized	51
All (minimum) botanical	14.3	To be finalized	368

Human exposure values were calculated from parametric estimations.

As noted previously, the preliminary fifth percentile values of the botanical datasets in Table 7 were obtained by a nonparametric estimation method, hence they were not transformed to human exposure values which are generally presented based on parametric estimation assuming normal distributions of the log(POD) values. These calculations will be done in the final phase of this work. The values for the Federated Class I and III were calculated using the same nonparametric method so the results can be compared to the botanical data. From this analysis, it is likely that the human exposure limit for the botanicals are ten times greater than that of the Cramer Class III of the Federated set and four times greater than that of the Class I. The comparison is depicted in Figure 5B.



## 4.0 Decision Tree & Framework

### 4.1 Botanical Decision Tree

There are series of steps in the process before the TTC decision tree is applied. They include: 1) identification of botanicals and compositions in the product; 2) Estimation of the exposure levels based on intended use; 3) Evaluation of available toxicity related to the test botanicals; 4) Identify the data gap to apply Botanical TTC approach. A sketch of the workflow for decision tree approach is summarized in Figure 6.

In Step 5 of this botanical workflow, the query substance is checked to determine whether it is not in the domain of applicability, namely whether the botanical substance should be excluded based on the recommended exclusion list, which is equivalent to cohort of concern chemicals in the Kores TTC tree (Kroes 2004). A substance determined to be within the domain of applicability is then passed to Step 6 where genotoxicity is considered. Genotoxic substances go to Step 8 where the 10  $\mu\text{g}$  threshold is applied to determine if it passes or requires further investigation. Nongenotoxic substances from Step 6 proceed to Step 7 where the botanical TTC threshold is applied to check whether the exposure is acceptable.

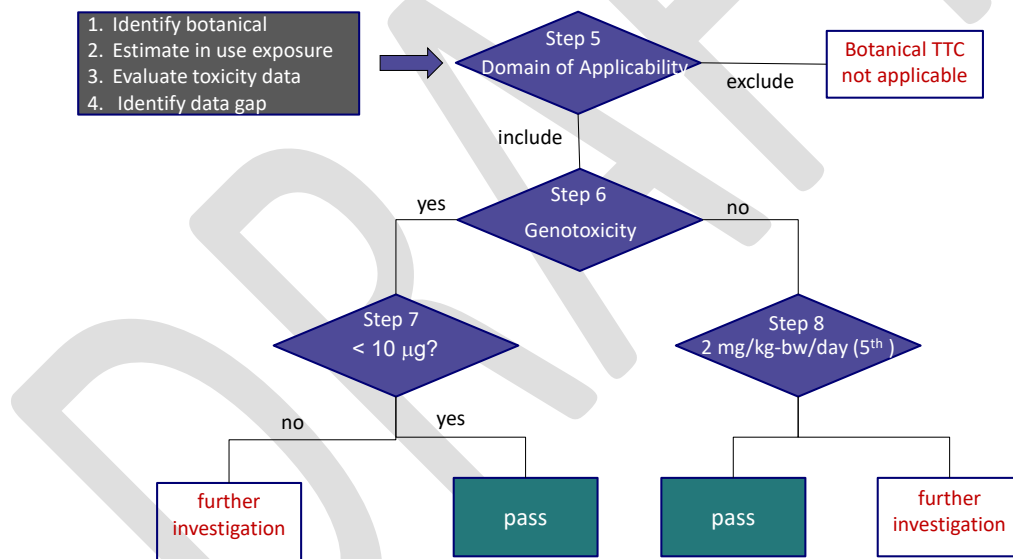
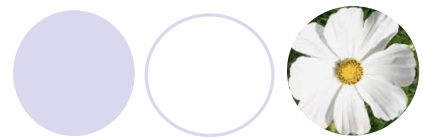


Figure 6. Proposed Botanical Decision Tree

Our analysis based on the preliminary dataset resulted in the fifth percentile for the minimum NOAEL distribution four times greater than the current cosmetics Class I threshold. Even if we take the most conservative approach and select the fifth percentile driven by two reproductive studies with plants containing phytoestrogens, we will still have 10 times higher than the current cosmetics Cramer Class III threshold. These tentative values are already much higher values proposed for botanical TTC approach previously (Kawamoto 2017). As we finalize our dataset, we will conduct parametric analysis to generate a set of robust thresholds that can be compared with other regulatory approaches.



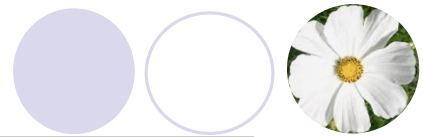
## 4.1 Exclusions When Applying the Botanical TTC Tree

TTC datasets often contain substances that fall under exclusion criteria. For example, the current Munro dataset contains polyhalogenated (although not dioxanes) and nitroso compounds. The exclusion as a domain of applicability does not mean that we need to take all the substances populating the lower end of the distribution. These extreme values in fact give the predictive conservatism that public use requires. As long as the data are robust and relevant, they can influence the threshold conservatively. However, it will be important to inform the users the list of substances and phytochemicals that are considered as concern.

- The botanical TTC approach should not be applied to abortifacient or reproductive influencers of phytoestrogens unless further rationale is provided to justify its use.
- A list of well-known toxins, toxic alkaloids, toxic essential oils, etc. should be prepared.
- Known abortifacient or fertility inhibitors should be listed. (Phytoestrogens known to affect estrous cycle, fertility, etc.)
- A few botanicals with confirmed carcinogenic potential (IARC or NTP) should be listed.

Table 8 Example of Possible Exclusion List (Preliminary Proposal)

Area of Concern	Primary Concern	Specific Examples
Carcinogenicity	IARC Group 1 (human carc.) Strong link to urinary tract cancer, kidney failure (nephropathy)	<i>Aristolochia</i> spp (e.g., <i>Aristolochia fangchi</i> Y.C. Wu ex S.K. Lee & L.K. Fu, Mu Tong, Snakeroot) Aristolochic Acids (AA I, II)
	IARC Group 1 (human carc.) Oral, pharyngeal, esophageal, and stomach cancer	<i>Areca catechu</i> (Areca nut) Betel Quid (Areca Nut)
	Potent DNA-alkylating agent, Gastric and urinary bladder cancer	<i>Pteridium aquilinum</i> Bracken Fern (Ptaquiloside)
	Liver damage and potential carcinogenicity Identified as genotoxic and carcinogenic compounds	Pyrrrolizidine Alkaloids. <i>Senecio</i> (ragworts), <i>Crotalaria</i> (rattleboxes), <i>Heliotropium</i> , and <i>Symphytum</i> (Comfrey)
Reproductive/ Developmental	Fertility loss and abortifacient causes	<i>Ruta graveolens</i> , <i>Mentha pulegium</i> , <i>Tanacetum vulgare</i> , <i>Tripterygium wilfordii</i> , <i>Carica papaya</i> , <i>Podophyllum peltatum</i>
	Teratogenicity e.g., fetal nicotinic acetylcholine receptors (nAChR)	<i>Lupinus</i> spp. Quinolizidine Alkaloid ( <i>Anagyrene</i> ); Piperidine alkaloid
Target organ toxicity	Hepatoxicity/DILI	<i>Teucrium chamaedrys</i> (Wall Germander) and <i>Teucrium polium</i> (Golden Germander). Teucrin A, Teuchmaedryn A



	Cardiac toxicity	<i>Aconitum</i> spp.; <i>Pausinystalia yohimbe</i> ( <i>Corynanthe johimbe</i> ). yohimbine, an indole alkaloid)
	Neuro toxicity	<i>Aconitum</i> spp.; <i>Atropa belladonna</i> ; <i>Datura stramonium</i> ; <i>Hyoscyamus niger</i>
...		

Work related to the establishment of the exclusion list for domain of applicability of the Botanical TTC approach is still in progress. by linking this list with our phytochemical section of the database is on-going at this point of the project.

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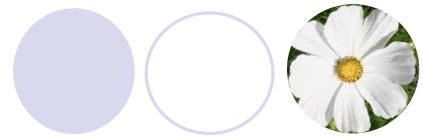
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