# Investigation into the effects of metabolism on the cytotoxicity of a subset of cosmetically-relevant compounds using an animal-product-free assay.

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## Introduction

The toxicity profiles of many chemicals are known to change following metabolism in the liver. This is often overlooked when using non-hepatic *in vitro* cytotoxicity assays and the effects of metabolic breakdown of compounds, either detoxification or the generation of toxic metabolic by-products, are seldom taken into account. Some toxicity models use metabolically competent cell lines or addition of exogenous metabolic components e.g. induced mammalian liver S9. However, these models rarely use human derived S9 even though this is widely available. Incorporation of metabolic functionality, in the form of human S9[1], into an animal-product-free cytotoxicity assay provides the basis of a more comprehensive assessment of cytotoxicity. The identification of raw materials that may yield cytotoxic metabolic-breakdown products, is useful in informing product safety.

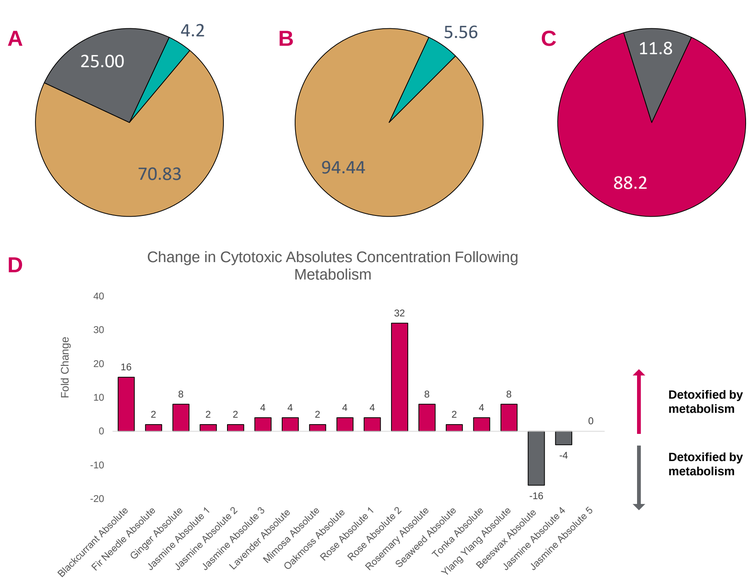
## Methods

The effect of metabolism (through addition of human liver S9 to the system) upon cytotoxicity to TK6 cells was investigated using a thiazole orange-based animal-product-free cytotoxicity assay for cosmetically-relevant, but often cytotoxic, ingredients that are widely used in cosmetics and fragrances. These chemicals were categorised based upon their structure or function[2].

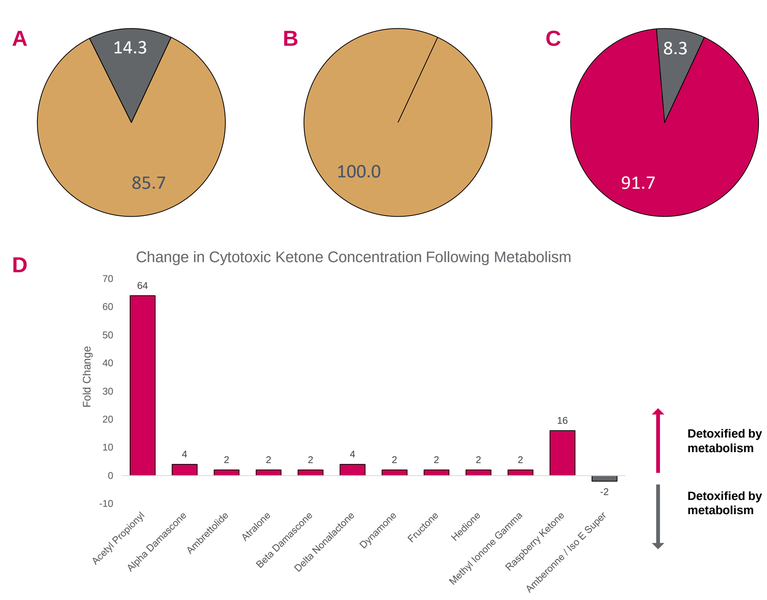
## Results

We have demonstrated that the majority of the toxicity profiles generated for cytotoxic raw materials in the ketone (100%), absolutes (94.44%), aldehyde (92.9%), dye/colourant (92.6%) and essential oil (76.4%) subgroups are altered by expanding the *in vitro* cytotoxicity test to encompass metabolic functionality.

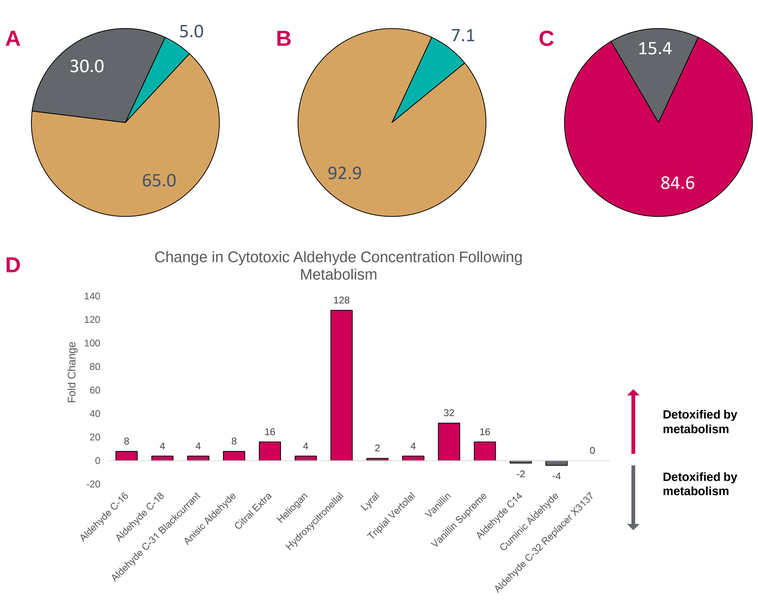
### Fig 1. Absolutes



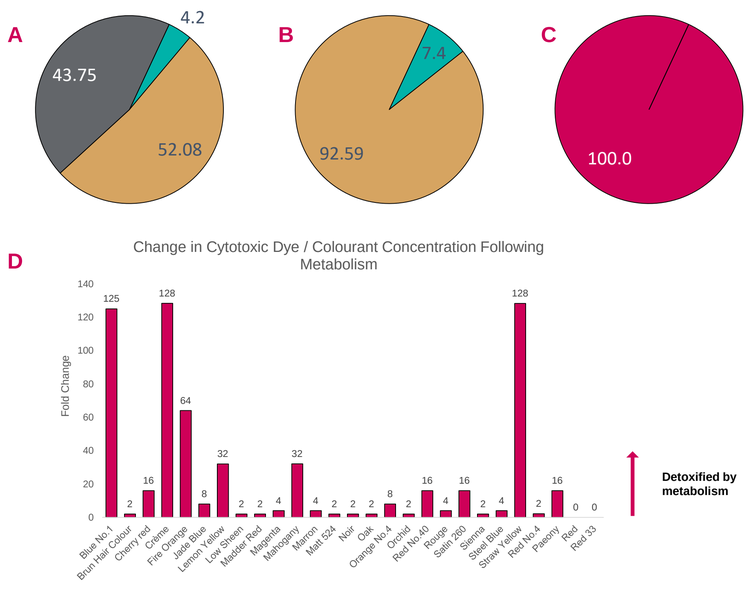

### Fig 2. Ketones



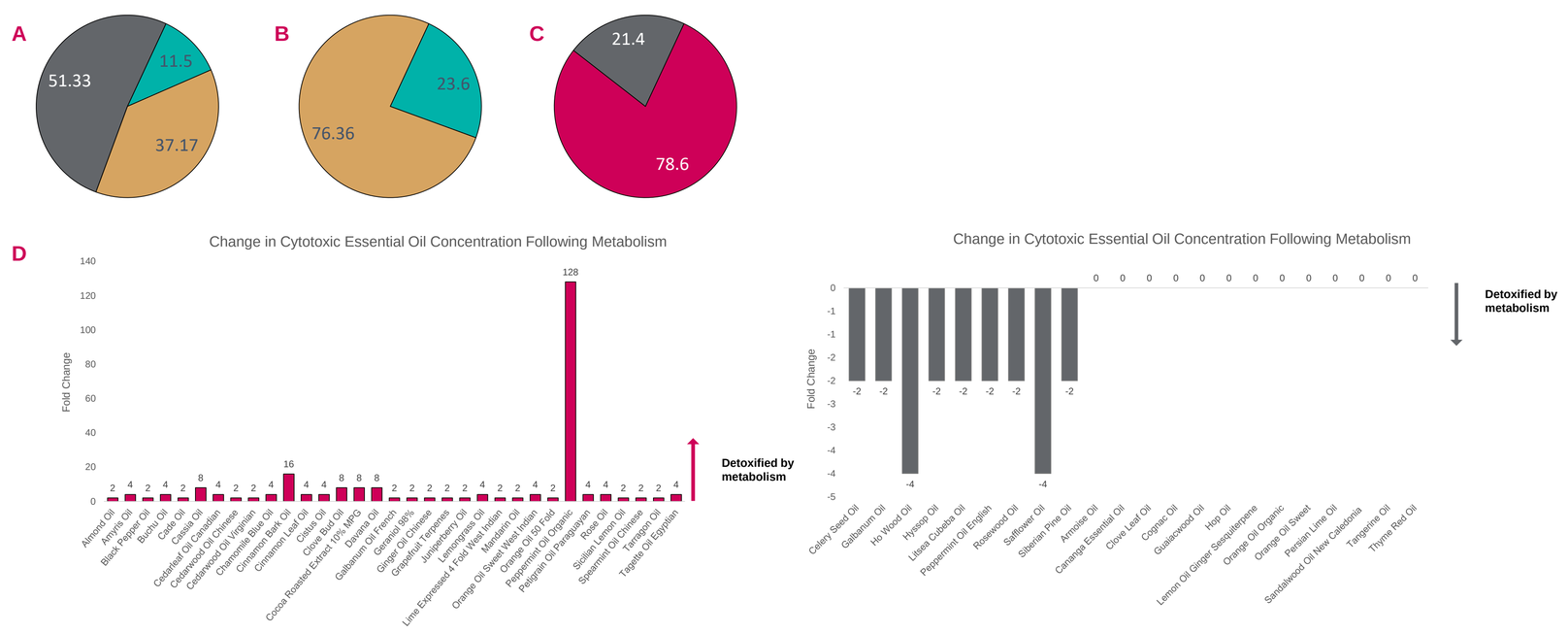

### Fig 3. Aldehydes




### Fig 4. Dye/Colourants




### Fig 5. Essential Oils




**A**: Gold = percentage of test substances that were cytotoxic in the test system and were altered by metabolism, Grey = percentage of test substances that were non-cytotoxic in the test system, Mint = percentage of test substances that were cytotoxic in the test system but were not altered by metabolism

**B**: Gold = percentage of test substances where cytotoxic concentration was modified by metabolism when included in the test system, Mint = percentage of test substances where cytotoxic concentration was not modified by metabolism.

**C**: Pink = percentage of cytotoxic test substances where concentration was modified by metabolism to yield a detoxification effect, Grey = percentage of cytotoxic test substances where concentration was modified by metabolism to yield cytotoxic breakdown products.

**D**: Bars represent the fold change in cytotoxic test substance concentration following modification by metabolism, Pink (positive values) = detoxification effect, Grey (negative values) = cytotoxic breakdown products yielded by metabolism, No bar (0 values) = cytotoxic test substance where concentration was not modified by metabolism.

## Discussion

To summarise the findings of this study, the cytotoxicity profiles of the majority of raw materials from 5 diverse groups were modified by metabolism when incorporated into an *in vitro* toxicity assay. Of the raw materials that were modified, those that were detoxified by metabolism and those that were modified to yield toxic metabolic breakdown products were able to be identified. Human S9 comprises a suitable metabolic system to be used in an *in vitro* acute toxicity assay. Incorporation of metabolism into *in vitro* acute toxicity screens is likely to improve the predictivity of the tests to better predict human health and reduce the incidence of false positive results. There is additional potential to use human cell based acute toxicity assays as screening tools in conjunction with emerging technologies such as Organ On A Chip[3]. that are paving the way for future *in vitro* / human *ex vivo* acute toxicity testing.

### References

1. Cox, J. A. et al., Mutagenesis. 31, 117-130 (2016)
2. Edwards, A. et al., Toxicology in vitro. (in press)
3. Zhang, B. et al., Nature Reviews Materials. 3, 257-278 (2018)