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Toxicological evaluation of an *Allium*-based commercial product in a 90-day feeding study in Sprague–Dawley rats

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ABSTRACT

Proallium AP® is a commercial *Allium* extract intended to be used in active food packaging as the antibacterial and antioxidant effects of some organosulfur compounds are well known. However, there is little information on its toxicity and the Scientific Committee on Food (UE) requires the safety assessment of substances used in food contact materials. Thus, the aim of this study was to conduct for the first time a subchronic oral toxicity study of Proallium AP® with groups of 10 males and 10 females Sprague–Dawley rats fed a diet containing 0, 25, 100, 400 mg/kg/d for 90 days. No treatment-related clinical signs or mortality were noted. Besides, no treatment-related effects with regard to any of the toxicological biomarkers considered were observed, including biochemical, haematological and histopathology parameters. In conclusion, the non-observed-adverse-effect-level (NOAEL) for Proallium AP® in rats was determined to be a dietary dose of 400 mg/kg/d under the present experimental conditions, a value 500-fold higher than the exposure derived from its potential use in active packaging.

1. Introduction

Scientific research has focused its interest on essential oils (EOs) from medicinal plants as natural sources of antimicrobial agents and antioxidants (Hassanien et al., 2015). EOs vary in odor and flavor, which are governed by the types and amount of their constituents (Tongnuanchan and Benjakul, 2014). *Allium cepa* (onion) and *Allium sativum* (garlic) exhibit marked antibacterial activity and antioxidant effects (Benkeblia, 2004; Ye et al., 2013); moreover, both of them are plant species containing organosulfur compounds (OSCs) with applications in active food packaging (Llana-Ruiz-Cabello et al., 2015a). Active food contact materials were defined in Regulation No 1935/2004 of the Europe Parliament and of the Council as "materials that are intended to extend the shelf-life or to maintain or improve the condition of packaged food. They are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food". The nature of active agents that can be incorporated is very diverse and includes organic acids, enzymes, bacteriocins, fungicides, natural extracts, ions and ethanol as well as the materials in which they are included, e.g., papers, plastics, metals or mixture of these materials (Danielli et al., 2008). Besides, more research is needed to develop cheaper, more easily applicable and effective packaging systems (Lee et al., 2015). Because of petroleum-based matrices used to develop food contact material take hundreds of years to decompose, biodegradable materials based on starch, such as polylactic acid (PLA) has attracted the attention of the industry (Debiagi et al., 2014). Thus, active films have been developed by extrusion using PLA incorporated with natural substances as antimicrobial compounds (Del Nobile et al., 2009).

In this sense, recently, the use of PLA films containing different percentages of an *Allium* spp extract (2%, 5% and 6.5% Proallium AP®) to be employed in the packaging of ready-to-eat salads have been proposed by our group (Llana-Ruiz-Cabello et al., 2015b). The developed films, especially those containing 5% and 6.5% Proallium AP® exhibit strong antimicrobial effects. Proallium AP® is a

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commercial product based on lipid-soluble OSCs, which are characteristic of the plant genus *Allium* spp, and it is used as flavoring of sauces, prepared meals and salads (Llana-Ruiz-Cabello et al., 2015b). Propyl thiosulphinate oxide (PTSO) is one of these OSCs obtained by the decomposition of initial components present in *Allium* spp. and it is incorporated in an inert commercial food-grade support (cyclodextrin) to produce Proallium AP®. The antimicrobial properties and the antioxidant role of PTSO have been previously demonstrated (Llana-Ruiz-Cabello et al., 2015c; Peinado et al., 2012, 2013; Ruiz et al., 2010). Other minoritary components of this *Allium* extract are dipropylsulfiide (DPS) and dipropyl disulfide (DPDS) (Llana-Ruiz-Cabello et al., 2015d). The incorporation of EOs in active food packaging can result in a higher human exposure and consequently, more research is needed to establish effective and safe concentrations of EOs (Seow et al., 2014).

In the case of active packaging, the EOs or natural extracts allowed to be used for this purpose have been not published so far in Europe. The Guidelines of the Scientific Committee on Food for safety assessment of substances used in food contact materials (European Commission, 2001), which have been recently updated (EFSA, 2008, 2015), establish the toxicological tests that have to be supplied for substances intended to be used in food contact materials. Thus, the core set includes at least 3 mutagenicity *in vitro* studies and a 90-day oral toxicity study in rodents. As far as we know, no components of *Allium* EO have been authorised for their use as active agent in food packaging, and their safety needs to be confirmed before their use in the food industry. After reviewing the scientific literature, in the case of OSCs of garlic/onion extracts, few toxicological studies have been described. The minor components, DPS and DPDS did not show any cytototoxic effects in Caco-2 cells (0–200 μM) nor mutagenic effects in the Ames test in the concentration range 0.1–200 μM (Llana-Ruiz-Cabello et al., 2015d). Moreover, Musk et al. (1997) observed significant changes in the number of chromosomal aberrations (CA), as well as in the sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells after diallyl sulfide (DAS) and diallyl disulfide (DADS) exposure. In relation to PTSO, the major OSC present in Proallium®, *in vitro* cytototoxic effects have been described in Caco-2 cells and HepG2 cells in a range between 350 and 415 μM, being 10-fold higher than the concentrations used in food packaging (Llana-Ruiz-Cabello et al., 2015c). Moreover, the potential mutagenicity/genotoxicity of PTSO has been recently assessed *in vitro* by a battery of genotoxicity tests at relevant concentrations according to its use in food packaging (0–50 μM). This compound exhibited a weak mutagenic potential on L5178YTk$^{+/-}$ cells after 24 h of treatment using the mouse-lymphoma assay (MLA), and an increase of binucleated cells

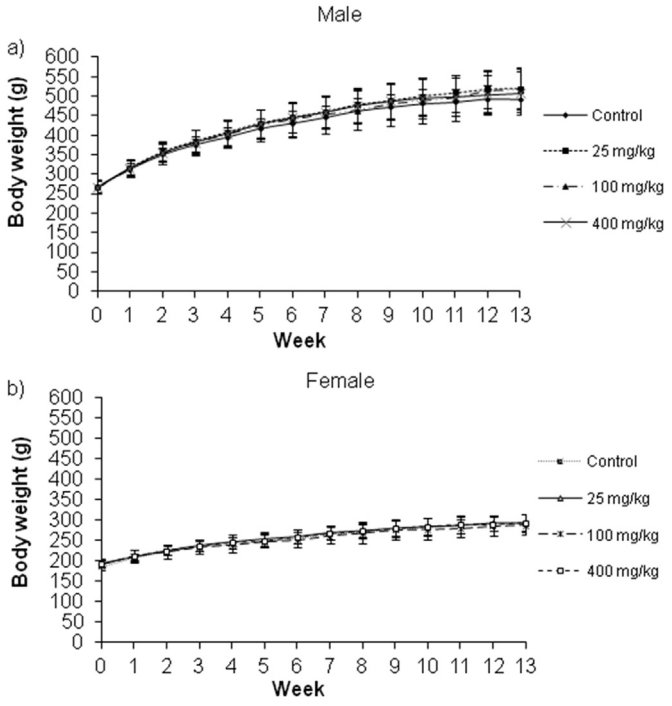

Fig. 1. Mean body weights of a) male and b) female rats exposed to Proallium AP® (25, 100 and 400 mg/kg/d) by oral route and control rats for 90 days.

with micronuclei (BNMN) frequency in presence of metabolic activation S9 (20 μM) (Mellado-García et al., 2015). With respect to *in vivo* experiments, Guyonnet et al. (2000) have demonstrated the effects of some OSCs (DAS, DADS, DPS and DPDS) on the activation of several mutagens in male Wistar rats, and various results were reported depending on the mutagen considered in each case. A preliminary *in vivo* acute study of PTSO has been conducted in rats orally exposed by gavage to establish the maximum tolerated dose (MTD) of this compound (Llana-Ruiz-Cabello et al., 2015c).

In relation to *in vivo* 90-day oral toxicity studies in rats of EOs with application in food industry (functional food ingredients, food packaging, etc.), only a few of them have been performed to assess their safety, such as: the ethanolic extract of *Artemisia dracunculus* L. (Ribnicky et al., 2004), the turmeric EO (*Curcuma longa*) (Liju et al., 2013), the ginger oil (*Zingiber officinale*) (Jeena et al., 2014), the peperina oil (*Minthostachys verticillata*) (Escobar et al., 2015), an

Table 1
Performance of Sprague–Dawley rats fed with different doses of Proallium AP® in the diet for 90-day. Values represent the mean ± SD of 10 rats/sex/group. Differences between control and treated groups for male and female rats were evaluated by Kruskal–Wallis test (K.W.) or by ANOVA test (F values).

Parameters	Male				Female			
	Group 1 (0 mg/kg/day)	Group 2 (25 mg/kg/day)	Group 3 (100 mg/kg/day)	Group 4 (400 mg/kg/day)	Group 1 (0 mg/kg/day)	Group 2 (25 mg/kg/day)	Group 3 (100 mg/kg/day)	Group 4 (400 mg/kg/day)
	N = 10	N = 10	N = 10	N = 10	N = 10	N = 10	N = 10	N = 10
Initial body weight (g)	268.3 ± 14.2	265.5 ± 13.4	266.4 ± 15.6	267.3 ± 18.0	185 ± 12.8	192.1 ± 8.1	190.1 ± 13.2	191 ± 10.6
	F(36.3) = 0.5511 p = 0.9827; N.S.				**F(36.3) = 0.7762 p = 0.5150; N.S.**			
Final body weight (g)	492 ± 25.3	518.5 ± 50.6	515.1 ± 57.2	508.1 ± 54.8	291 ± 17.9	291.6 ± 20.5	287.4 ± 25.5	293.1 ± 21.0
	F(36.3) = 0.5856 p = 0.6284; N.S.				**F(36.3)=0.1274 p = 0.9433; N.S.**			
Body weight gain	223.7 ± 19.27	253.0 ± 43.97	248.7 ± 48.60	240.8 ± 40.11	106.0 ± 16.03	99.5 ± 14.11	97.7 ± 17.49	102.1 ± 14.00
	KW = 2.028 p = 0.5666; N.S.				**F(36.3)=0.5311 p = 0.6639; N.S.**			
Total feed intake (g)	2094.6 ± 177.3	2194.4 ± 186.4	2112.6 ± 262.0	2137.9 ± 152.9	1491.2 ± 67.4	1531.9 ± 107.7	1501.3 ± 169.1	1549.3 ± 98.6
	F(36.3) = 1.065 p = 0.3759; N.S.				**KW = 1.587 p = 0.6622; N.S.**			
Feed conversion ratio	9.37 ± 0.66	8.82 ± 0.94	8,63 ± 0,93	9.01 ± 1.00	14.40 ± 2.57	15.64 ± 2.16	15.66 ± 1.86	15.36 ± 1.71
	F(36.3)=1.315 p = 0.2846; N.S.				**F(36.3) = 0.8005 p = 0.5018; N.S.**			

N.S.: Not Significant.