Case studies of supplement formulations with increased bioavailability

In this guide

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71. The following paragraphs outline three case studies of supplement compounds prepared as novel formulations. The case studies are intended to provide empirical pharmacokinetic outcomes of the mechanisms and physiological parameters discussed above and, specifically, to assess how novel formulations of supplement compounds may significantly affect plasma levels of active compounds. Some of these examples, therefore, may have toxicological implications.

72. The case studies are focused on controlled human trials in which novel and standard formulations are compared, rather than on *in vivo* and/or *in vitro* data. These studies are not exhaustive and attempt to provide an overview of realistic scenarios of how novel formulations of supplements may impact their bioavailability.

Case study 1: Liposomal vitamin C

73. A significant quantity of the novel formulations on the market appears to be liposomal formulations of vitamin C (see Table 3., above). Liposomal vitamin C supplements therefore provide an informative case study for investigating how novel formulations might impact supplement bioavailability and pharmacokinetics with potential implications for consumers.

74. Due to its potential role in cancer therapy at high doses, a significant amount of attention has been given to the pharmacokinetics of vitamin C. Vitamin C (ascorbate/ascorbic acid) is a hydrophilic compound with complex pharmacokinetics. Its bioavailability is limited by saturable transport mechanisms in the small intestine, its absorption follows a non-linear process, and body levels are dependent on current intakes. Some authors have argued that encapsulation of vitamin C in liposomes may result in a more prolonged release thereby increasing its uptake (Duconge *et al*., 2008). Liposomal encapsulation may also bypass saturable uptake mechanisms via direct transport into the lymphatic system (Duconge *et al*., 2008).

75. Liposomal formulation of vitamin C, therefore, is not designed to increase its solubility in the GIT, as with lipophilic molecules, but to bypassing its transport rate-limited absorption. This observation underscores the importance of investigating novel/alternative formulations of supplements on a case-by-case

basis.

76. Despite its significant presence in the market, however, there are only a handful of controlled studies investigating the oral bioavailability of liposomal vitamin C in humans. A couple of these studies were performed with small sample sizes. These studies and their conclusions are summarised below. The majority of these studies (4/6) were conducted in the last two years, indicating an emergent research interest in formulating supplements with increased bioavailability.

77. In an early study from 2008, Hickey *et al*. investigated the oral pharmacokinetics of standard and liposomal vitamin C. The study contained only two participants, one male and one female. Both subjects received 5 g vitamin C in standard formulation, the female received 5 g and 36 g in liposomal formulation, and the male received 20 g and 36 g in liposomal formulation. These larger doses were administered to test hypotheses about maximum blood levels achievable from oral dosing. Liposomes were composed of phosphatidylcholine (Hickey *et al.,* 2008).

78. In the female subject, the concentration-time curves of plasma vitamin C levels were similar for standard and liposomal formulations (5 g), albeit, with a slightly delayed Tmax (from 100 to approximately 200 minutes). In the male subject, 20 g liposomal vitamin C produced a concentration-time curve with a broader profile than that observed with a 5 g dose of standard vitamin C. In both subjects, administration of 36 g liposomal vitamin C led to plasma levels of approximately 400 µM, higher than that suggested by the NIH (National Institutes of Health) to be possible from oral dosing at the time of the study, and higher than that achieved via oral dosing or 5 g liposomal vitamin C in the present study. Although pharmacokinetic parameters (Cmax and area under the curve; AUC) were not reported for the 36 g liposomal dose, The concentration-time curve suggested that the liposomal vitamin C resulted in slower onsets to peak levels, and broader profiles, than the 5 g standard dose. The authors argued that these findings indicated a more sustained absorption of liposomal vitamin C owing to the physiological handling of liposomes (Hickey *et al.,* 2008).

79. Davis *et al*. (2016) compared the oral pharmacokinetics of liposomal encapsulated and non-encapsulated vitamin C in 11 older (53±2 years) overweight adults (34.1±1 kg/m^2 BMI). The vitamin C dose was 4 g. Liposomes were made with “mixed natural phospholipids” classified as Generally Recognised as Safe (GRAS) ingredients (GRAS is a designation applied to food ingredients by the United States Food and Drugs Administration. It is a designation that a chemical or substance added to food is considered safe by experts under the

conditions of its intended use and is therefore exempt from review as an additive).

80. At two-, three-, and four-hours post-administration, plasma vitamin C levels were significantly higher with liposomal vs. non-liposomal vitamin C (*p* 0.001). The AUC0-4h (the area under the time concentration curve up to 4 hours post administration) was 1.4-fold greater with liposomal vs. non-liposomal vitamin C (10.3±0.9 vs 7.6±0.4 mg/dL h), indicating that oral bioavailability of vitamin C was increased by liposomal formulation. Plasma levels achieved by oral dosing with either standard or liposomal formulation were significantly lower than that achieved by intravenous administration (IV) at all time points (*p*0.001). IV vitamin C achieved a Cmax of approximately 27 mg/dL, compared to approximately 3.5 mg/dL for liposomal and approximately 2 mg/dL for standard vitamin C (*p* values not reported) (Davis *et al*., 2016).

81. Łukawski *et al*. (2020) studied the oral pharmacokinetics of liposomal vitamin C compared to unencapsulated vitamin C in 20 healthy participants. Ten participants received a standard formulation of vitamin C, whilst 10 received a liposomal form. The liposomes used in this study were formulated from soybean phosphatidylcholine. Following administration of 10 g vitamin C, the maximum blood concentration reached (Cmax) was higher in those receiving the liposomal vs. non-liposomal formulation (303 µM vs. 180 µM) and the time taken to reach the maximum concentration (Tmax) was longer, by approximately one hour, from 96 to 180 minutes. The half-life was also longer: >6 hours compared to 4 hours. The authors concluded that the results “indicate that the presence of liposomes enhances bioavailability of vitamin C.” The authors further suggested that the increased bioavailability of liposomal vitamin C was related to protection from degradation inside the GIT which provided a sustained reserve of the compound for absorption.

82. Gopi and Balakrishnan (2021) compared the oral bioavailability of liposomal and non-liposomal vitamin C in 24 healthy adults in a cross-over design trial. Participants received 1 g of vitamin C. Tmax was unaffected by formulation (approximately 3.5 hours), whereas Cmax was increased with the liposomal formulation (5.2 versus 1.2 mg/dL). The AUC0-24h analysis also demonstrated an increase with liposomal vitamin C (55.9 versus 31.5 mg•h/dL), whilst half-life was increased from 12.4 to 19 hours with the liposomal formulation.

83. Joseph *et al*. (2021) designed and evaluated the oral pharmacokinetics of a multilamellar surface engineered liposomal vitamin C formulation (in the form of calcium ascorbate). Liposomal surfaces were engineered/modified by

impregnation into a fenugreek galactomannan hydrogel in a powder form. All ingredients used were “food grade” and the process was designed to stabilise liposomes from harsh physiological conditions, thereby enabling sustained and increased absorption.

84. Fourteen healthy participants were administered 1 g of vitamin C either in liposomal or non-liposomal forms in a cross-over design. The liposomal formulation resulted in significantly higher plasma vitamin C levels over 12 hours (p0.05). Liposomal vitamin C in tablet and capsule form resulted in a Cmax of 282 and 273 µM, respectively, versus 52 µM for unformulated control. The half-life was also increased from 3.6 hours with unformulated vitamin C to 8.5 and 7.6 hours for tablet and capsule forms of liposomal vitamin C, respectively. The AUC0-12h was increased by approximately 7-fold with the liposomal versus non-liposomal vitamin C preparation. The authors suggested that the larger increase in the AUC observed in their study versus that seen in other liposomal vitamin C studies was due to the enhanced stability of liposomes embedded in a fibre matrix.

85. Jacob *et al*. (2021) also evaluated the oral pharmacokinetics of a fibre-reinforced liposomal vitamin C preparation. The fibre was of turmeric origin. Eight participants were administered 150 mg of vitamin C in liposomal or standard formulations, in a cross over design. Liposomal vitamin C increased the Cmax from 1.2 mg/dL to 6.7 mg/dL and increased the AUC0-24h by 5.9-fold. Like Joseph *et al*. (2021), the authors suggested the enhanced bioavailability of fibre-reinforced liposomal vitamin C was due to the stability of the formulation under physiological conditions.

86. In summary, liposomal preparations of vitamin C appear to increase oral bioavailability as determined by pharmacokinetic studies. The effects of liposomal vitamin C on the AUC0-n and Cmax in the studies discussed above are summarised in Table 4.

Table 4. Summary of effects of liposomal vitamin C on AUC and Cmax versus non-liposomal preparations.

Study	**Cmax positive fold difference**	**AUC0-n positive fold difference**
Davis *et al*. (2016)	n.r.	1.4

Łukawski *et al*. (2020)	1.7	1.8
Gopi and Balakrishnan (2021)	2.4	1.8
Joseph *et al*. (2021)	5.4	7
Jacob *et al*. (2021)	5.4	5.9

n.r.: not reported. Fold differences in Cmax and AUC0-n were calculated by the Secretariat from the original publications.

Case study 2: Curcuminoids

87. Due to their poor oral bioavailability, novel formulations designed to enhance the oral bioavailability of curcuminoids have been extensively studied. However, it should be noted that “while a large number of such formulations are developed in academia and as garage projects, only a few of them are available on the market in one form or another.” (Jamwal, 2018). Nonetheless, from analysis of the scientific literature, grey and white literature, curcumin appears to be a supplement for which novel formulations designed to increase oral bioavailability are in the more advanced stages of formulation research, design, commercialisation, and marketisation (compared to, for instance, CBD). The following paragraphs, therefore, relate primarily to studies investigating the pharmacokinetics of commercially available curcuminoid formulations.

Review by Jamwal, 2018

88. Jamwal (2018) published a review of studies investigating the pharmacokinetics of different curcuminoid formulations and calculated their relative oral bioavailability compared to unformulated curcuminoids. Table 5 provides an overview of Jamwal’s (2018) review and indicates the relative bioavailability of the various formulations. Relative oral bioavailability values were calculated by Jamwal (2018) using the following formula:

(Relative bioavailability = AUC formulation X Dose control)/ (AUC control X Dose formulation).

Table 5. Summary of studies investigating effects of curcuminoid formulation on oral bioavailability. Adapted from Jamwal (2018).

Characterisation	**Relative oral bioavailability (positive fold change, from Jamwal, 2018)**	**Reference**
Phytosomal Emulsion-based (curcumin, soy lecithin, microcrystalline cellulose)	48	Cuomo *et al*., (2011)
Solid lipid curcumin particles	100	Gota *et al*., (2010)
Fenugreek soluble fibre-based delivery system	15.8	Im *et al*., (2012)
Dispersed micronized curcuminoids.	9.7	Madhavi and Kagan, (2014)
Micronised curcumin	9	Schiborr *et al*., (2014)
Liquid micelles	185	Schiborr *et al*., (2014)
Water-dispersible curcumin complex – Polyvinylpyrrolidone and cellulose based	136.3	Jäger *et al*., (2014)
Turmeric essential oil formulation	6.9 [see corrigendum to Jamwal, 2018]	Antony *et al*., (2008)

γ-cyclodextrin-based formulation	85	Purpura *et al*., (2018)
Colloidal nanoparticles	15.9	Sasaki *et al*., (2011)

89. Overall, the novel formulations summarised by Jamwal (2018) increased the oral bioavailability of curcuminoids compared to administration of unformulated curcuminoids ranging between 6.9 and 185-fold. Of the formulations reviewed, liquid micelles provided the greatest increase in relative bioavailability (185-fold).

90. However, there are important limitations in comparing across these studies. In the first instance, most of the studies reported in Table 5 administered different doses of unformulated vs. formulated curcumin and thus required dose-normalisation to extrapolate relative oral bioavailabilities. Some studies indicated that curcuminoid pharmacokinetics are non-linear (Kocher *et al*., 2015), suggesting that this method may misrepresent fold-changes in bioavailability between preparations (Flory *et al*., 2021).

91. There was significant variation in the preparative and analytical methods used for detection of plasma curcuminoids and their metabolites. Some of the studies measured levels of free curcuminoids, whereas others quantified conjugated curcumin. Conjugated curcumin is the primary metabolite present in plasma; however, it is less pharmacologically active than the free compound. There were also differences in which metabolites were analysed (curcumin, demethoxycurcumin - DMC, bisdemethoxycurcumin - BDMC, tetrahydrocurcumin - THC), and there is ongoing debate about the relative impact of these metabolites on toxicity. Differences were also apparent in the detection and quantification methods; whilst some studies used high-performance liquid chromatography (stand-alone), others used liquid chromatography-mass spectrometer-based determination.

92. Other important differences related to the clinical trial design including fasting status and food intake after administration of the curcuminoids, which may have important effects on curcumin absorption. There were also differences in the race/ethnicity composition and gender balance of the various cohorts. Some studies have reported sex-differences in the absorption of curcuminoids which is important to consider.

Other studies

93. Several studies not reported by Jamwal (2018) have also investigated the pharmacokinetics of curcuminoid formulations designed to increase oral bioavailability in human subjects. The following paragraphs summarise some of the key findings from these studies. The studies included here were those comparing the pharmacokinetics of oral curcuminoids in standard preparations versus novel formulations in healthy human subjects.

Lipid-based formulations

94. Kocher *et al*., (2015) studied the effects of micellarisation on curcumin pharmacokinetics in healthy volunteers The effects of the adjuvant phytochemicals sesamin, ferulic acid, naringenin, and xanthohumol were also investigated. The study included 23 healthy volunteers administered 98 mg total curcuminoids and was designed as a cross-over trial with one-week washout periods between subsequent treatments.

95. Curcumin, DMC, and BDMC levels were quantified from plasma. The oral bioavailability of total free curcumin was increased by formulation with phytochemicals, as micelles, and as micelles with phytochemicals by 8-fold, 88-fold, and 73-fold, respectively (comparing the AUC to the control group administered unformulated curcumin). Micellar formulation also increased the AUC of curcumin metabolites DMC and BDMC by 848 and 159-fold, respectively, relative to unformulated curcumin. Overall, micelles were effective at increasing curcumin absorption, and this effect was not further increased by adjuvant phytochemical micelles.

96. Asher *et al*. (2016) used a crossover study design to compare the pharmacokinetics of unformulated curcumin with that of a curcumin-phosphatidylcholine formulation in 12 healthy subjects. Although the physicochemical properties of the phosphatidylcholine complex used were not reported, the Secretariat has assumed this is likely to be a colloidal dispersion of curcumin-phosphatidylcholine. The authors examined plasma and colorectal tissue levels of curcuminoids after administration of 1000 mg unformulated curcuminoids or 385 mg of curcumin-phosphatidylcholine complex once daily for 7 days. Plasma samples were taken immediately prior to the last dose, and then 11 times over 24 hours following the last dose.

97. Tmax was shorter for phosphatidylcholine-curcumin complex versus unformulated curcumin (64 minutes versus 216 minutes for curcumin,

respectively). Dose-adjusted AUC0-24h analysis demonstrated that curcumin, DMC, and BDMC (conjugated forms) plasma levels were increased 8.8, 2.9, and 3.0-fold, respectively, with phosphatidylcholine-curcumin versus unformulated curcumin. Curcumin (conjugated and free), DMC (conjugated only), and BDMC (conjugated only) were also detected in rectal mucosa tissue, but their levels were not different between the formulations.

98. Panda *et al*. (2019) investigated the oral pharmacokinetics of curcumin formulated as 'Curene®' ®' versus two reference curcumin formulations – standardised 95% curcuminoids and CP-01, a curcumin formulation containing turmeric volatile oil. Curene® is a proprietary curcumin formulation that, according to the authors, forms an "emulsion similar to liposomes upon contact with the aqueous environment [of] intestinal fluids" (Panda *et al*., 2019), suggesting a S(M)EDDS-like mechanism.

99. Three grams of each curcumin formulation were administered to 12 healthy male subjects split into 3 groups (4 subjects per formulation) and 10 blood samples were collected from point of administration up to 24 hours post-administration. Cmax of free curcumin from the Curene®-curcumin formulation was significantly higher than for control curcumin (1546 vs. 86 and 190 pg/ml for standardised curcuminoids and CP-01, respectively; *p*0.05), with no change in Tmax. Compared to standardised curcuminoids and CP-01, AUC0-24h was increased by 31 and 14-fold, respectively, (from 207 and 445 pg•h/ml, respectively, to 6303 pg•h/ml; *p*0.05).

100. Briskey *et al*. (2019) compared the oral pharmacokinetics of a novel surfactant, polar-lipids, and solvent-based dispersion curcumin formulation to that of a standard curcumin preparation in 7 healthy human subjects. The so-called LipiSperse® technology is added to an aqueous suspension of curcumin crystals. The surfactant and lipid-based product then forms a coat around the curcumin crystals, coating them, preventing agglomeration, and increasing aqueous solubility.

101. Curcumin formulated with LipiSperse® led to increases in the Cmax and AUC0-6h for curcumin, DMC, and BDMC compared to standard curcumin. In a crossover trial with 5 healthy subjects, curcumin Cmax was increased 3-fold, from 215 to 691 ng/mL (*p*0.05) and total AUC0-6h was increased 2.0-fold*p*0.05). Tmax was unchanged between preparations (1 hour). In a parallel study design with 8 healthy subjects, curcumin total AUC0-6 was 2.3-fold higher in those receiving Lipisperse® curcumin and Cmax was increased by 4.4-fold (151 vs 658 ng/mL; AUC and Cmax *p*0.05).

102. Fança-Berthon *et al*. (2021) compared the oral pharmacokinetics of unformulated curcumin, liquid micellar, phytosomal, and dried-colloidal curcumin formulations in 30 healthy subjects. Different doses of each formulation were used and in accordance with the supplier's daily recommended doses (1500 mg unformulated curcumin, 1000 mg phytosomal curcumin, 1000 mg liquid micellar curcumin, 300 mg dried-colloidal curcumin). The authors argued that this approach provided meaningful data that could be applied to exposures expected through the real-world use of these products.

103. For non-dose adjusted analysis, the AUC0-24h of total curcuminoids from the liquid micellar formulation were significantly higher than the group receiving unformulated curcumin (control group; *p*0.0001). When AUC0-24h was adjusted for dose, plasma curcuminoids were also significantly increased with liquid micellar, dried-colloidal, and phytosomal curcumin formulations (136, 73, and 13 ng•h/ml/mg, respectively versus 3.7 ng•h/ml/mg for the control group; *p* 0.0001 for each).

104. A 2022 study by Kanae *et al*. investigated the pharmacokinetics of orally administered curcumin in four different formulations: unformulated curcumin extract, curcumin mixed with squalene, curcumin mixed with docosahexaenoic acid and solid lipid curcumin particles (SLCP). Pharmacokinetics of all four preparations were compared separately in 10 Japanese individuals (5 male and 5 female) >20 years and 65 years of age. A 7-day washout period was observed between trials (Kanae *et al*., 2022).

105. Higher doses of unformulated curcuminoids (260 mg, control group) were administered than for formulated curcuminoids (SLCP: 88mg, squalene: 82 mg, docosahexaenoic acid: 79 mg) and pharmacokinetic parameters were normalised to curcuminoid doses for the various formulations. Conjugated curcuminoids were detected after glucuronidase/β-sulfatase pre-treatment of plasma samples. The Tmax of curcumin was not significantly changed between the formulations (*p*>0.05), but those of DMC and BDMC were significantly shorter with SLCP, docosahexaenoic acid, and squalene formulations compared to the control group (*p*0.05).

106. Plasma levels of curcumin and total curcuminoids were higher with the novel formulations at all time points (1 – 8 hours), whilst plasma levels of DMC and BDMC were higher at earlier time points (1 -2 hours), compared to control. The dose-normalised AUC0-8h of curcumin was significantly increased in all the novel formulations compared to the control: 0.43, 0.45, and 0.55 ng/ml.h/mg for solid lipid particles, squalene, and docosahexaenoic acid, respectively, versus

0.19 ng/ml.h/mg for control (p0.01, p0.05, and p0.01, respectively).

107. The dose normalised Cmax of curcumin was also significantly higher for all the novel preparations versus unformulated curcuminoids: 0.09, 0.09 and 0.12 ng/ml/mg for solid lipid particles, squalene, and docosahexaenoic acid, respectively, versus 0.05 for control (p0.05, p0.05, and p0.01, respectively). This amounted to a relative increase of curcumin absorption of 2.2, 2.3 and 2.8-fold for solid lipid particles, squalene, and docosahexaenoic acid preparations, respectively. The AUC0-8h of DMC and BDMC were not different for the novel preparations versus control, whereas their Tmax was significantly shortened for all the preparations (p0.05). The only sex difference observed was a significantly higher dose normalised Cmax for DMC in men administered the standard curcuminoid preparation (p=0.04).

Dispersion technologies

108. Sunagawa *et al*. (2015) investigated the oral bioavailability of Theracurmin® (182 mg), a colloidal submicron-particle formulation of curcumin, in healthy human subjects compared to liposomal (Meriva®; 152 mg) and micronised curcumin mixed with turmeric essential oils (BCM-95; 279 mg). Theracurmin® is a proprietary technology, and an earlier study investigating this formulation (Sasaki *et al*., 2011) was included in Jamwal's (2018) review, who calculated an increase in relative oral bioavailability over unformulated curcumin of 15.9-fold. Theracurmin® is composed of "curcumin dispersed with colloidal submicron-particles" (Sunagawa *et al*., 2015). This colloidal dispersion is based on the water-soluble polysaccharide gum ghatti that has emulsifying characteristics and can increase the water solubility of lipophilic compounds. To produce Theracurmin®, curcumin powder was added to a gum ghatti water solution, ground by a wet grinding mill, and dispersed by a high-pressure homogeniser (Sunagawa *et al*., 2015).

109. The Sunagawa et al., (2015) study was designed as a 3-way crossover with nine subjects with a 7-day washout period between administration of the different formulations. Theracurmin® resulted in a higher curcumin Cmax (287.2 ng/mL) than BCM-95 and liposomal curcumin of10.7 and 5.6-fold, respectively(p 0.05). AUC0-6h for Theracurmin® was significantly higher than that of BCM-95 and liposomal curcumin by 16.1 and 5.6-fold (p0.05), respectively, whilst the AUC0-24h was 11 and 4.6-fold higher, respectively (p0.05).

110. Panda *et al*. (2021) studied the oral bioavailability of a "novel dispersible" curcuminoid extract compared to a standard curcumin extract. The

extract under study was the proprietary CURCUGEN an oleoresin-based turmeric formulation that derives its dispersible properties from turmeric-native polar resins, turmeric essential oils, and turmeric polysaccharides. This formulation preserves the “food-state” ratio of curcuminoids (i.e., the natural ratio of DMC and BDMC), as opposed to standardised curcumin extracts.

111. The oral bioavailability of CURCUGEN was studied in a 2-way crossover trial in 17 healthy male subjects. Plasma levels of free and total curcumin, total DMC, BDMC, curcuminoids, and THC were quantified up to 24 hours post administration. CURCUGEN significantly increased levels of free and total curcumin, and all the curcumin metabolites studied (p0.05). Based on AUC0-24h, plasma levels of all curcuminoids analysed were significantly increased (p0.05): free curcumin (39-fold), total curcumin (50-fold), DMC (44-fold), BDMC (47-fold), total curcuminoids (53-fold), and THC (31-fold).

Comparative studies

112. Flory *et al*. (2021) argued that, owing to non-linear pharmacokinetics, comparing oral bioavailability of curcuminoid formulations administered at different doses by using the relative AUC method is flawed. A number of studies discussed in the previous sections utilised the relative AUC method, and this may therefore be a consideration when interpreting those studies.

113. Flory *et al*.’s (2021) comparative study compared the effects of different curcuminoid formulations on oral bioavailability using the same administered dose of total curcuminoids between formulations. They compared the pharmacokinetics of seven curcumin formulations designed to increase oral bioavailability with that of native curcumin: micellar, γ-cyclodextrin formulation, phytosomal, submicron-particle, with adjuvants (piperine), with turmeric oil, and liposomal. Preparations were administered at identical doses of curcumin (207 mg) in 12 individuals (6 male, 6 female) per group in a cross-over design.

114. Plasma levels of curcumin were measured over 24 hours. Only the administration of micellar curcumin and γ-cyclodextrin-formulated curcumin led to increases in the AUC0-24h (57-fold and 30-fold, respectively). Micellar curcumin also significantly increased the AUC0-24h relative to γ-cyclodextrin-formulated curcumin (p0.05). Females had significantly higher AUC0-24h than males after uptake of micellar curcumin (p0.05). Phytosomal and submicron-particle curcumin led to non-significant increases in the AUC0-24h of 7.5- and 6.5-fold, respectively.

115. *In vitro* digestive assays demonstrated that sub-micron particles, micellar, and γ-cyclodextrin-formulated curcumin had the highest digestive stabilities (109%, 102% and 73%, respectively). In those same assays, solubility and micellisation efficiency were highest for micellar and γ-cyclodextrin formulations; micellar and γ-cyclodextrin curcumin had solubilities of 80% and 33%, respectively, whilst micellisation efficiency was 55% and 23%, respectively (calculated as “mass curcumin in mixed micellar fraction/mass curcumin in raw material”). Bioaccessibility studies in Caco-2 cells (a human colorectal model) suggested that apparent permeability did not differ between the formulations.

116. Overall, Flory *et al*., (2021) argued that the increased oral bioavailability of micellar and γ-cyclodextrin-formulated curcumin preparations resulted from increased pre-digestive stability and post-digestive solubilisation in gastrointestinal conditions. Increased transport across the epithelium or inhibition of biotransformation and/or epithelial efflux pumps had no effects on oral curcumin bioavailability.

117. The study by Flory *et al*. (2021) suggests that comparing relative oral bioavailability of curcumin formulations administered at different doses may be misrepresentative. There are also limitations, therefore, in directly comparing between different studies that used different doses. The magnitude of this effect is likely to be exacerbated when there are large differences in doses, and when analysis of plasma curcuminoids is close to or at the limit of detection.

118. Despite these methodological limitations, the literature suggests that novel formulations of curcumin in lipid-based and dispersion systems have the potential to increase oral bioavailability of curcumin and its metabolites. Table 6 provides a summary of the curcumin formulations that led to increased bioavailability in the above studies. The table lists the increase in bioavailability as defined by increased fold changes in Cmax and AUC0-n for curcumin only, as calculated in the respective publications.

Table 6. Summary of curcumin formulations increasing curcumin AUC and Cmax in healthy human studies. Preparations that did not affect AUCs as part of the same study are not included in the table.

Formulation	**Cmax positive fold difference (curcumin)**	**AUC0-n positive fold difference (curcumin)**	**Study**

SLCP	2	2	Kanae *et al*. (2022)
Micelle	216	88	Kocher *et al*. (2015)
Micelle	84	37	Franca-Berthon *et al*. (2021)
Phytosomal	1.2[a]	9	Asher *et al*. (2016)
Phytosomal	203	57	Flory *et al*. (2021)
Aquesome®	18	31	Panda *et al*. (2019)
LipiSperse®	3	2	Briskey *et al*. (2019)
Dried colloidal	23	20	Franca-Berthon *et al*. (2021)
Squalene-curcumin preparation	2	2	Kanae *et al*. (2022)
Docosahexaenoic acid-curcumin preparation	2	3	Kanae *et al*. (2022)
Colloidal submicron	11	11	Sunagawa *et al*. (2021)

Dispersible form (CURCUGEN)	25	50	Panda *et al*. (2021)
γ-cyclodextrin curcumin	56	30	Flory *et al*. (2021)

AUC fold differences were calculated by the secretariat based on presented data. Where available, fold differences were calculated from total curcumin plasma levels, and from the AUC for the longest defined time period. SLCP: solid lipid curcumin particles; n.r.: not reported; [a] values are not dose-normalised and are from administration of 4000 mg standard and 400 mg phytosomal curcumin.

Case study 3: Cannabidiol

Background

119. Cannabidiol (CBD) is a highly hydrophobic molecule known to be of relatively low oral bioavailability, with reports suggesting an average of approximately 6% (Millar *et al*., 2020). Moreover, a large degree of inter-individual variation exists in the absorption of CBD (Millar *et al*., 2018) and absorption is modified by feeding state (Silmore *et al*., 2021; Mozaffari *et al*., 2021).

120. Preparation of CBD also affects its bioavailability. For instance, Williams *et al*. (2021) demonstrated that oral bioavailability differed between five different CBD formulations. A preparation comprising 5% CBD concentrated liquid (containing medium-chain triglyceride (MCT) oil, gum arabic, and citric acid in reverse osmosis water) evoked the shortest Tmax, highest Cmax, and largest AUC0-4h, whilst CBD powder suspended in reverse osmosis water had the lowest oral bioavailability.

121. The most bioavailable CBD preparation in Williams *et al*.'s (2021) study was formulated with medium-chain triglyceride (MCT) oil, citric acid, and gum arabic in reverse osmosis water. The authors suggested that the presence of gum Arabic and medium-chain triglyceride (MCT) oil may have aided CBD solubilisation in the GIT and therefore enhanced absorption.

122. However, whilst formulation type can affect the bioavailability of CBD, questions remain as to how this translates into the supplement market and the

precise products to which consumers might be exposed. CBD is widely consumed as a supplement in the UK and is available in a variety of formulations, for instance as oils, tinctures, capsules, in beverages, and in food. It is potentially misrepresentative, therefore, to speak of a ‘standard’ formulation of CBD.

123. However, a preliminary analysis conducted by the Secretariat of 51 CBD supplements available from the online market suggests a large portion of CBD supplements (29/51) are formulated with medium-chain triglyceride (MCT) oil as a carrier. The most common delivery method is oral oil drops: 25 out of the 51 products are formulated as oral oil drops, 19 of which use medium-chain triglyceride (MCT) oil as a carrier, with the remainder using either hemp seed oil or rice brain oil. Gummies are the second most common formulation (12/50) whilst oral sprays and capsules comprise the remainder.

124. One entry for micellar CBD was found. The proprietary formulation of this product was NovaSOL®, and the pharmacokinetics of curcumin formulated in this way have been studied in control settings (see above section). There were no other indications for CBD formulated in ways to increase oral bioavailability on a preliminary search of the online market, but a further general internet search identifies several possible products on the market.

Studies investigating the oral pharmacokinetics of CBD formulations

125. Alternative formulations have been designed to increase oral CBD bioavailability and alter its pharmacokinetic profile. Owing to potential application of CBD to treat symptoms of disease, a large amount of this research has been based on development for pharmaceutical indications. Unlike curcumin, however, there are currently only a few clear examples of novel formulation products available on or destined for the supplement/nutraceutical market (or to wholesalers/white label who supply this market).

126. However, some of the CBD formulations in development as academic and/or pharmaceutical projects have used food-grade ingredients to design preparations that could conceivably be adopted by supplement manufacturers. Additionally, owing to the regulatory status of CBD, some of these applications and/or products occupy a grey area between the pharmaceutical and supplement markets, and their penetrations into either space is possible.

127. Based on this reasoning, the following paragraphs summarise key studies investigating formulations of CBD with increased bioavailability in human

subjects. The studies are selected to indicate possible formulations that might increase bioavailability and offer a ‘horizon scanning’ perspective on formulations that, owing to their formulation characteristics, might conceivably penetrate the CBD supplement market in the future.

128. Hobbs et al. (2020) investigated the relative oral bioavailability of two commercially available CBD formulations: ‘water-soluble’ and ‘lipid-soluble’ powders in 10 healthy subjects in a randomised parallel arm study. Volunteers were administered 30 mg CBD, which was suggested to be a ‘standard’ dose based on available products. The water-soluble powder had Cmax of 2.82 ng/mL and a Tmax of 90 min. The abstract to this study states that the water-soluble powder was approximately 4.5-fold more bioavailable than the lipid-soluble form.

129. De Prá *et al*. (2021) prepared a self-emulsifying drug delivery system (SEDDS) designed to increase the oral delivery of CBD. As described in the above section, SEDDS are lipid-based preparations of active ingredients formulated with lipids, surfactants, and/or co-surfactants that self-emulsify upon contact with the aqueous conditions of the GIT to form mixed micelles and potentially increase absorption of lipophilic compounds (Pouton and Porter, 2008). The CBD SEDDS was prepared with polyoxyl 40 castor oil as the emulsifier and polyethylene glycol 400 as the co-emulsifier, both of which are food-grade ingredients.

130. The De Prá *et al*., (2021) study also investigated the effects of partially hydrolysed long-chain triglycerides (GML) as an excipient on the oral bioavailability of CBD. GML is composed of a mixture of mono-, di-, and triglycerides, which may improve the solubility of CBD via promoting mixed micelle formation. CBD formulated with medium-chain triglyceride (MCT) oil was used as the reference preparation. *In vitro* digestion studies demonstrated that the majority of CBD from the SEDDS remained partitioned in the aqueous phase post-digestion, suggesting a complete solubilisation under these conditions. Only a low percentage of CBD from the other two preparations, however, was recovered in the aqueous phase.

131. The preparations were investigated in a controlled trial comprising 11 (analysed) subjects. The trial was designed as a three-arm crossover study with 7-day washout periods between administration of subsequent formulations. These human pharmacokinetic studies demonstrated that SEDDS CBD formulation led to an increased Cmax and AUC0-12h versus the medium-chain triglyceride (MCT) formulations (2 and 1.5-fold, respectively). GML preparation also increased the Cmax and AUC0-12h by 1.9-and 1.3-fold, respectively. Both the SEDDS and GML formulations also decreased the Tmax of plasma CBD levels (1.7 and 1.6 hours,

respectively, versus 4.3 hours). The authors concluded that the “bioavailability of [CBD] is significantly influenced by the physicochemical characteristics of [excipient] lipids, the length of the fatty acid chain, and its susceptibility to digestion.”

132. Knaub *et al*. (2019) also investigated the effect of a SEDDS on the oral pharmacokinetics of CBD. Their SEDDS was based on the VESIsorb® technology, a proprietary SEDDS system for which commercial ubiquinol formulations are already available on the market. The VESIsorb® SEDDS is comprised of “food emulsifiers, edible vegetable oils and fatty acids.”

133. Bioavailability was studied in sixteen healthy volunteers who were administered 25 mg CBD either formulated with medium-chain triglyceride (MCT) oil or with the SEDDS in a cross-over study design. SEDDS-CBD significantly increased oral bioavailability as indicated by increases in the Cmax and AUC0-24h of 4.4- and 1.7-fold, respectively (p0.0001 and p=0.0021). Tmax was also reduced from 3h to 1h with the SEDDS versus medium-chain triglyceride (MCT) oil CBD.

134. In interpreting their findings, Knaub *et al*., (2019) suggested that the increased oral bioavailability of CBD formulated with a SEDDS is due to the formation of droplets that solubilise CBD in the GIT that deliver the molecule to enterocytes for absorption. Moreover, lymphatic transport, which bypasses the first-pass effect known to limit oral bioavailability of CBD, may also play a role.

135. Izgelov *et al*. (2020) compared the oral bioavailability of 90 mg CBD powder (no dissolution vehicle), CBD dissolved in sesame oil, and CBD formulated in a self-nano-emulsifying drug delivery system (SNEDDS) in a three-way crossover trial in 12 healthy subjects. The SNEDDS was composed of ethanol, soy lecithin, and surfactants (Tween 20, Span 80, and Kolliphor RH40).

136. CBD formulated in lipid-based systems was more bioavailable than CBD powder: Cmax was increased 22.5-fold and 17.5-fold with the SNEDDS and sesame oil CBD preparations, respectively, whilst AUC0-24h was increased approximately 8-fold for each formulation compared to the CBD powder. The SNEDDS also reduced Tmax and its associated variability (2 hours, versus 4 hours and 8.4 hours for sesame oil CBD and powder CBD, respectively). Sub-analysis of the sesame oil CBD time-concentration curves suggested the existence of two absorption behaviours in different groups of subjects; an ‘early’ and ‘delayed’ absorption population. Izgelov *et al*., (2020) suggested that the SNEDDS CBD formulation provided a less variable absorption profile owing to the consistent

physicochemical parameters of the resultant emulsion compared to the sesame oil CBD preparation.

137. Patrician *et al*. (2019) investigated the oral bioavailability of a novel CBD formulation called ‘TurboCBD’ in a double-blinded, placebo controlled cross-over design with 12 participants. 45 mg or 90 mg CBD was administered. circulating CBD levels were higher with the TurboCBD 90 mg group at both 90 and 120 minutes compared with the 90 mg control (p0.05). Total area under the curve tended to be higher with TurboCBDTM 90 mg compared with 90 mg standard dose but did not reach statistical significance (10,865 ng/mL vs. 7,114 ng/mL; p=0.088). The authors concluded that TurboCBD had a higher bioavailability than a standard CBD preparation.

138. A pilot study from Blair (2020) reported on the pharmacokinetics of liposomal CBD in a cross-over trial with 15 healthy subjects compared to a control non-liposomal formulation. Ten mg of CBD were administered, and CBD blood levels were measured at 1-hour post-ingestion. Mean plasma CBD levels were higher with administration of liposomal versus non-liposomal CBD (1.77 ng/ml versus 0.24 ng/ml). Moreover, whilst CBD in plasma was detected in 6/15 participants administered non-liposomal CBD, it was detected in all of those (15/15) receiving liposomal CBD.

139. In summary, several bioavailable formulations of CBD appear to be emerging in academic research, and a number of these are tied to commercial interest for supplement formulation. A summary of the effects of the CBD formulations on the Cmax and AUC in the studies discussed above is presented in Table 7.

Table 7. Effects of CBD formulations on AUC and Cmax in healthy human subjects.

Bioavailable formulation	**Reference formulation**	**Cmax positive fold difference**	**AUC0-n positive fold difference**	**Study**
Water-soluble CBD	Lipid-soluble CBD	n.r.[a]	4.5	Hobbs *et al*. (2020)
GML CBD	MCT CBD	1.8	1.3	De Prá *et al*. (2021)

SEDDS CBD	MCT CBD	2.0	1.5	De Prá *et al*. (2021)
SEDDS CBD (VESIsorb®)	MCT CBD	4.4	1.7	Knaub *et al*. (2019)
Sesame oil CBD	Powder CBD	17.5	8.3	Izgelov *et al*. (20200
SNEDDS CBD	Powder CBD	22.5	7.6	Izgelov *et al*. (2020)
Liposomal CBD	'non-liposomal' CBD	7.4[b]	7.4[b]	Blair (2020)

AUC and Cmax fold differences were calculated by the secretariat based on presented data. AUC fold differences were calculated from the longest defined time period. [a] Abstract only retrieved. [b] from baseline-1 hour only (i.e., 'Cmax' by definition, but only one time point tested).

Toxicology studies with novel supplement formulations

140. The increased bioavailability of supplements formulated in novel ways as discussed above may have important toxicological implications. As EFSA (2018) state in their 'guidance for risk assessment on nanotechnologies': "If nanoencapsulates function as intended…there will be increased bioavailability (systemic exposure) of the encapsulated material. This represents a potential concern since health-based guidance values are currently set based on the external rather than the internal dose and may no longer provide an appropriate level of protection to the consumer." HBGVs, therefore, may need reassessing in light of specific formulations and relative bioavailabilities. This also suggests that exposure assessments based on standard formulations may potentially underestimate exposure from novel formulations.

141. In addition to increases in bioavailability, novel supplement formulations may alter toxicological profiles through other toxicokinetic parameters such as alterations in tissue distribution, or via changes in physiology not reported in the studies reviewed above. Furthermore, there may be formulation specific toxicological effects that cannot be extrapolated from toxicology studies using standard formulations of a given compound / supplement. For instance, it is reported that administration of vitamin C may enhance iron absorption which may be of concern for individuals with hemochromatosis or heterozygous for this disorder (EVM, 2008). However, the mechanism of vitamin C enhanced iron absorption occurs through the chelation of ferric iron which increases the solubility of the latter (Lynch and Cook, 1980), an effect which may be less relevant for encapsulated vitamin C formulations which are less able to physically interact with iron (example formulated by the Secretariat). These considerations suggest the need for case-by-case evaluation of specific formulations with respect to their potential toxicological implications beyond effects on bioavailability.

142. An initial review of toxicological effects which may be related to increased bioavailability, or specific to novel formulations of the compounds reviewed above was conducted using a literature search. The full results of this literature search, including the search strings used, number of retrieved results, and brief summaries of *in vitro* and *in vivo* studies are presented in appendix 1. This was not designed as a comprehensive review of toxicological effects related to novel formulations but intended as a preliminary scoping exercise to guide future assessments and/or to identify data gaps.

143. No studies investigating toxicological effects of novel formulations of vitamin C or CBD were identified. This reflects the lack of knowledge regarding the safety of novel formulations in general. Thus, although toxicological data exists on these active compounds, there are significant data gaps as to how these toxicological profiles may be altered when they are formulated in the ways discussed above.

144. Several studies investigating the toxicology of novel curcumin formulations in experimental systems and/or human subjects were retrieved. The majority of these studies investigated the toxicity of novel curcumin formulation in *in vitro* and/or *in vivo* systems, and three studies investigated effects in human subjects. Owing to the scope and aims of the current discussion paper, the *in vitro* and *in vivo* preclinical studies are summarised briefly in appendix 1 and include cytotoxicity assessments in a number of primary cells and cell lines and

toxicological studies in rats and mice. A number of these studies might be of interest for inclusion in future discussion papers. The studies in human subjects are summarised below.

Curcumin

Human studies

145. Storka *et al*. (2015) investigated the safety and tolerability of a liposomal curcumin in healthy human subjects in a randomised dose escalation study. Subjects were administered liposomal curcumin intravenously at either 10, 20, 40, 80, 120, 180, 240, 320 or 400 mg/m^2. Because of adverse reactions to mean red blood cellular volume and the formation of echinocytes only two subjects received 400 mg/m^2. Red blood cell echinocyte formation was dose-dependent, detectable at a threshold dose of 120 mg/m^2, without clinical symptoms, transient, and fully recoverable at 6 hours post infusion. Increases in mean red blood cellular volume were observed in two subjects administered 400 mg/m^2 and did not associate with markers of haemolysis but did associate with increased venous serum lactate concentrations (maximum of 3.7 mmol/L versus normal range of ≤ 2.2 mmol/L). Twenty five subjects also experienced at least 1 adverse event; there were 49 adverse events in total, 11 of which were moderate and 38 of which were mild. Based on the dose-dependent, transient, and reversible effects on echinocyte formation and increases in mean red blood cell volume, the authors concluded that “a single intravenous dose of liposomal curcumin is considered safe up to a dose of 120 mg/m^2 when infused over a period of 2 hours.”

146. In another dose escalation study, Greil *et al*. (2018) assessed the safety, tolerability, and efficacy of liposomal curcumin in 32 patients with locally advanced or metastatic cancer. Liposomal curcumin was administered intravenously weekly for 8 weeks, and the dose was increased from 100 mg/m^2 over 8 hours to 300 mg/m^2 over 6 hours. Twenty-six patients successfully completed dose-escalation without dose-limiting toxicity. However, the number of adverse events related to the treatment increased with doses at 300 mg/m^2. One patient receiving 300 mg/m^2 developed haemolysis, three patients treated with this dose displayed haemoglobin decreases without signs of haemolysis, whilst one patient exhibited definite haemolysis. Out of a total of 143 adverse events, 34 were considered related to the treatment and the remainder to underlying disease. Two of these events, facial oedema, and anaemia, were considered serious. Echinocytes were also observed in one patient. Although adverse events

were observed, this was a study population with advanced cancer disease, many of whom had exhausted other lines of treatment and all of whom were taking other medication at the time of the study, and therefore these events may not be generalisable to other populations.

147. It should be noted that the two above studies (Storka *et al*., 2015 and Greil *et al*., 2018) administered liposomal curcumin via intravenous infusion. This may have resulted in plasma levels that would not be achieved by oral dosing alone.

148. Kocher *et al*. (2016) investigated the safety of micellar curcuminoids in moderately hyperlipidaemic individuals. Subjects consumed 294 mg of micellar curcuminoids or placebo per day for 5 weeks. Neither blood lipids, nor markers of inflammation, glucose and iron homeostasis, or liver enzymes differed between curcuminoid and placebo interventions. The authors concluded the intervention was safe.