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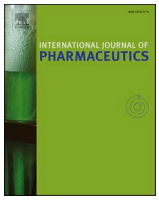

Taste assessment for paediatric drug Development: A comparison of bitterness taste aversion in children versus Naïve and expert young adult assessors

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ABSTRACT

Medicines for children often taste bitter, presenting a significant challenge to treatment compliance. However, most studies on paediatric drug development rely on adult volunteers for sensory research, and the level of expertise required from these assessors is unclear. This study aimed to address this gap by investigating perceived bitterness aversion to taste strips impregnated with different concentrations of quinine hydrochloride in 439 school-aged children. Expert (n = 26) and naïve (n = 65) young adult assessors evaluated quinine solutions as well as taste strips, for methodological bridging purposes. All assessors differentiated the aversiveness of the taste strips in a dose dependent manner. Younger children aged 4–8 years had difficulty discriminating higher bitter concentrations, whereas pre-adolescents 9–11 years and naive adults showed better discrimination at the top of the scale. Naive assessors showed similar bitter perception as children. However, the results were slightly different between strips and solution in adults. These findings highlight the key role that adult panels can play in paediatric formulation development. Taste strips show promise as a safe and pragmatic tool for sensory pharmaceutical evaluations, though further studies are warranted to establish the relationship between age and hedonic taste perception using compounds with diverse physicochemical and sensory qualities.

1. Introduction

Perceiving the unpleasant taste of an active pharmaceutical ingredient (API) is a significant barrier that hinders patients' willingness, and indirectly, ability to take their medicinal products. Indeed, taste is one of the core components that encompasses palatability, the overall appreciation of an oral medicinal product, alongside characteristics such as smell, aftertaste and mouthfeel (European Medicines Agency 2013). Ensuring formulation palatability is especially pertinent when developing medicines for children, whose well-established preference for sweet tastes and dislike for bitter stimuli reflects their basic biology (Mennella and Bobowski 2015). Although this evolutionary trait has facilitated the survival of our species through protection from toxic materials (Breslin 2013, Wooding et al., 2021), it can have detrimental consequences in the context of modern medicine where many APIs exhibit a bitter or aversive taste.

Adults often have a higher tolerance towards taking unpalatable medicines given their appreciation of the resulting health benefits; however, children can face more difficulty in overcoming taste aversions. In a survey of US paediatricians, 90.8% reported unpleasant medication taste a key barrier to patient compliance (AAP Division of Health Policy Research 2001), while in a UK study, almost a third of children reportedly refused medicines, with taste and texture being significant predictors of this outcome (Venables, Batchelor et al. 2015).

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In a recent large survey of almost 700 European children (median age 14 years), the most frequent response to "*Why do you find some of the medicines difficult to take?*" was disliking the taste, as reported by 63.7% of respondents (Nordenmalm, Kimland et al. 2019). This was almost twice as frequent as the second most common problem, pain during administration, reported by 32.5% of respondents. Studies have also shown medication taste to be a common barrier to adherence with antiepileptics (Gutierrez-Colina, Smith et al. 2018), antibiotics (Baguley, Lim et al. 2012) and antimalarials (Banek, DiLiberto et al. 2021).

It is vital to determine the taste characteristics of an API as early as possible during drug development. Identifying the need for and suitability of different taste-masking approaches facilitates the development of formulations with favourable organoleptic properties to improve patient acceptability, and accordingly, therapeutic outcomes. The European Medicines Agency's (EMA) seminal guideline on paediatric pharmaceutical development highlights that early palatability data can be acquire from dedicated adult panels or literature; however, patient acceptability of the final proposed medicinal product should preferably be studied in children themselves as an integral outcome of clinical studies (European Medicines Agency 2013). Research studies with children that aim to capture palatability data can be limited by fewer test samples and simpler tasks in line with their cognitive abilities. While a range of in vivo and in vitro methods for early taste evaluation exist today (Mohamed-Ahmed, Soto et al. 2016, Al-Kasmi, Al Rahal et al. 2018, Guedes, Marques et al. 2021), the use of dedicated sensory panels with adult assessors remains the gold standard practice (Clapham, Bennett et al. 2021).

Sensory analysis is "*the science involved with the assessment of the organoleptic attributes of a product by the senses*" (International Organization for Standardization 2016), and is most widely used for consumer research in the food and drinks industry. Nevertheless, the principles can provide considerable benefit to non-food sectors including pharmaceutical product development. Sensory evaluation involves assembling a dedicated panel of human volunteers, considered as the "measuring instrument", and applying standardized principles of experimental design and statistical analysis to obtain quantitative and qualitative responses, including hedonic tests for liking, product preference tests, descriptive analysis, and sensory profiling. The International Organization for Standardization (ISO) and ASTM International (formerly known as American Society for Testing and Materials) are organisations which have published numerous standards dedicated to sensory evaluation.

Participants recruited for such studies, deemed sensory assessors, can be classified according to their experience and any methods employed in their selection and training, as per normalised sensory analysis vocabulary (International Organization for Standardization 2016). A naïve assessor is "*a person who does not meet any particular criterion",* while an expert assessor is *"a selected assessor with a demonstrated sensory sensitivity and with considerable training and experience in sensory testing, who is able to make consistent and repeatable sensory assessments of various products"*. Different assessor types can confer their own unique advantages, for example, expert assessors are trained to objectively analyse and characterise products and communicate sensory perceptions using harmonised terminology, whereas naïve assessors may better represent the product's target population.

Taste detection from medicines arises from the interaction of the dissolved API, and indeed excipients, with taste receptors in the mouth and throat. The "swirl and spit" technique is a widely used taste assessment methodology, where a liquid solution or suspension of the test substance is moved around the mouth to cover the palate, before being spat out and appraised. This approach can be beneficial in minimising the risk of adverse effects related to the ingestion of drug substances while still providing critical taste data. For obvious health and safety reasons, it would be unethical to expose children unnecessarily to APIs, even using this type of methodology. Although adult sensory assessors are employed to guide the development of paediatric formulations, very little is currently known about the degree of similarities and differences in taste sensitivity and aversion to bitter stimuli among these populations. Therefore, understanding if and how taste data from adults can be interpreted or extrapolated to adequately aid paediatric drug product development is essential.

"Taste strips" (TS), impregnated with sample tastants and applied directly to the tongue, present an attractive option with potential to be adapted for use in pharmaceutical sensory evaluation studies. This methodology was originally developed and validated in adults as a mean to assess gustatory sensitivity in clinical settings (Mueller, Kallert et al. 2003, Landis, Welge-Luessen et al. 2009), and commercial testing kits are now available on the market. Assessing the minimum concentrations at which subjects can perceive and differentiate specific taste qualities, including bitter, sweet, sour, salty and umami, supports the diagnosis of taste disorders. Moreover, this methodology has been used successfully with children and adolescents as young as 6 years old (Overberg, Hummel et al. 2012, Hill, Beach et al. 2016, Sauer, Ohla et al. 2017, van den Brink, IJpma et al. 2021). Accordingly, there is merit in understanding whether TS can be adapted as a novel methodology to safely and easily measure children's taste perceptions of APIs to support the development of palatable paediatric medicines.

Quinine, in one of its more soluble salt forms, is a well-established, model bitter substance that is commonly used to evaluate this basic taste sensation. It is recommended as a reference material to train sensory assessors in the detection and recognition of bitter stimuli (International Organization for Standardization 2012). A proof-of-concept study with naïve adult assessors demonstrated that TS impregnated with quinine hydrochloride (QHCl) elicited comparable responses to aqueous QHCl solution samples (Georgopoulos, Keeley et al. 2019). The present study aimed to compare sensory responses to bitter stimuli from school-aged children (or paediatric assessors) and adults using this same TS methodology. A secondary outcome was to expand and further validate the initial proof-of-concept work by comparing responses to QHCl TS and aqueous solutions (AS) with both naïve and expert adult sensory assessors. Collectively, these research outcomes address important methodological questions related to the level of expertise required when using human volunteers for preclinical sensory research studies to inform paediatric drug development. Moreover, it aims at proposing a safe and efficient means of conducting out of lab (OOL) sensory panels.

2. Material and methods

2.1. Preparation of taste strips (TS) and aqueous solutions (AS)

QHCl was purchased from Fagron (Fagron, Newcastle upon Tyne, UK) in the UK and from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) in France. The TS were grade MN713 filter paper sheets (Macherey-Nagel, Germany) cut to 8 cm × 1 cm in size and manually impregnated with a 30 μl QHCl solution to achieve the corresponding milligram amount per strip. The TS were left to dry in the dark for at least 2 h at room temperature, and then stored for up to 1 week under the same conditions before use. The AS samples were stored in the dark for 24 h at room temperature before the sensory panels took place.

Both TS and AS samples were prepared at increasing concentrations

Table 1
Quantity (mg) of quinine hydrochloride (QHCl) impregnated on taste strips, and QHCl aqueous solutions (mg/ml) tested.

Taste Strips (TS)		Aqueous Solutions (AS)	
Sample label	**QHCl impregnated on strip (mg)**	**Sample label**	**QHCl in solution (mg/mL)**
TS1	0.015	AS1	0.005
TS2	0.044	AS2	0.015
TS3	0.132	AS3	0.044
TS4	0.396	AS4	0.132

as shown in Table 1. During prior TS development research, in vitro drug release studies had demonstrated that approximately one-third of QHCl released from the strips over a 10 s timeframe, equivalent to the time that participants would evaluate the strips on the tongue (Georgopoulos, Keeley et al. 2019). Therefore, the AS samples were prepared accordingly for each strip, considering that one-third of the quantity of QHCl impregnated on the TS released in 1 mL of saliva.

2.2. Sensory panel studies

A paediatric sensory panel study with primary school children aged 4–11 years old was completed by the University College London (UCL) research team in the UK, while adult sensory panels with naïve and expert assessors were conducted by the Ecole de Biologie Industrielle (EBI) team in France. The design of each panel study is summarised in Table 2. Approvals were sought from local research ethics committees (REC) (EBI study ID: EuPFI 19,062,020 and UCL study ID: 4612/026).

2.3. Study participants and informed consent

For the paediatric panel, all pupils aged 4 to 11 years at the primary school were invited to take part in the research study. Specially designed, age-adapted information sheets were disseminated to all children and their adult parents/caregivers, and both informed caregiver consent and participant assent were recorded for each child who volunteered to take part.

All young adult panellists in France were EBI students aged 18 to 25 years who provided informed consent before participating. For the first panel with naïve sensory assessors, participants were not required to meet any precise criterion for selection and did not undergo any training or have any particular prior knowledge of sensory testing. In contrast, for the second panel with expert assessors, all participants were trained over twelve sessions during which they assessed the intensity of different tastant solutions, including bitterness (QHCl) according to EBI internal procedures adapted from ISO 8586 (International Organization for Standardization 2012). This was performed to ensure panel discrimination and reproducibility.

Table 2
Study designs for three sensory panels conducted with adult and paediatric assessors in the UK and France.

Participants	Samples Tested	Procedure	Outcome Measures	Panel Setting
Naïve paediatric sensory assessors aged 4 – 11 years	4 QHCl TS	Each TS evaluated on the tongue for 10 s. No compulsory neutralisation between samples.	Bitterness aversion rated on a 5-point facial hedonic scale and continuous visual analogue scale (VAS), both anchored from "It's ok" to "It's horrible".	East London Primary School, UK
Naïve young adult sensory assessors aged 18 – 25 years	4 QHCl TS 4 QHCl AS	Each TS evaluated on the tongue for 10 s. AS (10 mL) evaluated for 10 s using the swirl and spit technique. Standardised neutralisation completed between samples.	Bitterness aversion rated on a 5-point facial hedonic scale and continuous VAS, both anchored from "It's ok" to "It's horrible".	Dedicated sensory analysis test rooms, France
Expert (trained) young adult sensory assessors aged 18 – 25 years	4 QHCl TS 4 QHCl AS	Each TS evaluated on the tongue for 10 s. AS (10 mL) evaluated for 10 s using the swirl and spit technique. Standardised neutralisation completed between samples.	Bitterness intensity rated immediately on an 11-point scale from 0 "Not Bitter" to 10 "Bitter".	Dedicated sensory analysis test rooms, France

2.4. Sensory evaluation procedures

For evaluation of the TS, both adult and child assessors were instructed to place the strip on the middle on the tongue ("like a lollipop") and close their mouths for 10 s, before removing and rating. This was a way to standardise the method, to make it as easy and intuitive as possible for children. Only the adult naïve and expert assessors evaluated the QHCl AS samples. The TS and AS samples were tested during separate panels on different days. Panellists were instructed to swirl each 10 mL solution all around the mouth, including under the tongue, against the cheeks and on the palate, for 10 s before spitting out and rating. The different solution concentrations were presented to the panellists in a given order according to a Williams design plan, a Latin square, high-crossover design to achieve balance and maximise comparisons with the fewest number of subjects.

Naïve paediatric and adult assessors reported hedonic bitterness aversion, while the trained expert assessors reported bitterness intensity (the magnitude of the perceived sensation) using the scales depicted in Fig. 1. As per ISO guidelines, pictorial scales with stylised faces illustrating expressions are recommended for hedonic tests with children to cater to their more limited reading and comprehension abilities (International Organization for Standardization 2003). Continuous visual analogue scales (VAS) have also demonstrated reliability as self-report measurement tools in children, therefore both scales were implemented in this study for methodological evaluation. The unipolar scales were designed starting from a neutral value, "It's ok", to an extreme dislike anchored with the term "It's horrible"; these terms were selected as scale anchors to ensure they could be easily understood by young children. The corresponding pictorial faces were adapted from a 5-point pain rating scale (Chambers and Craig 1998). For analysis purposes, the facial scales were coded sequentially from 1 (neutral face) up to 5 (negative face) in line with increasing levels of aversion; however, the numbers were not depicted on the scale presented to assessors. Results from the VAS were analysed as a numerical value on a 100-point scale from 0 (It's ok) to 100 (It's horrible).

In the adult taste panels, a compulsory standardised neutralisation procedure to cleanse the palate was completed before and after testing the solutions or strips. This involved swirling a sample of plain water in the mouth and then spitting it out. Unleavened bread was also consumed to help remove any residual taste sensations, and a 3-minute interval was observed between testing each TS or AS sample. Due to time constraints and the limitations in attention spans innate to conducting research with children, there was no inter-sample neutralisation in this panel. However, plain water was available for the children to consume between testing each TS, if they wanted to. Once finished, children were offered dried fruits to end the experiment on a nice note and help with residual bitterness, if any. Bottled water unleavened bread, and dried fruits were bought from local supermarkets in each country.

2.5. Statistical analysis

Statistical analyses were performed using XLSTAT® software (sensory version 2021, Addinsoft, New York) and R version 4.1.1 (2021–08-10) and package ggplot2 version 3.3.5. According to Guide in uncertainty in measurements (GUM), the Shapiro-Wilk's test for normality

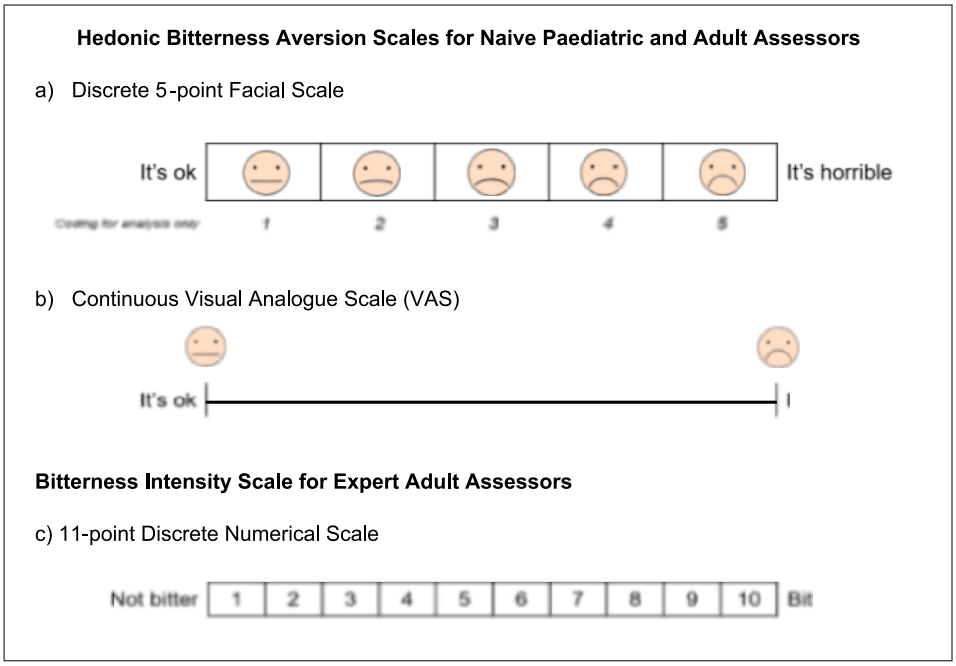

Fig. 1. Response scales used to capture bitterness aversion with naïve children and adult assessors and bitterness intensity with expert adult assessors.

was performed to assess distribution. Following visual inspection of the data, where normality was not observed, a non-parametric Friedman test was run. This method is based on rank analysis that enables comparison of $p > 2$ paired samples.

For all tests, the α risk value was set at 0.05, and was compared to the p-value obtained for each statistical analysis. Following Friedman Tests, the Nemenyi's procedure was used for multiple pairwise comparisons between products. The Nemenyi procedure is a post-hoc test developed to classify samples into groups that are significantly distinct from each other. Finally, Spearman's correlation was performed to compare responses to samples using the two different scales.

3. Results

3.1. Participant Demographics

The characteristics of the study population are summarised in Table 3. In the paediatric panel, there were on average 55 child participants for each individual age group.

3.2. Hedonic 5-point facial scale vs. Continuous VAS

Fig. 2 shows the correlation between the responses from the paediatric assessors (n = 439) for all taste strips evaluations captured on the 5-point facial scale and continuous VAS. A Spearman's rank-order correlation was run to assess the relationship between the two data collection scales. There was a statistically significant strong positive correlation observed between the scales for both paediatric assessors and naïve adults assessors ($r2 > 0.9$; $p < 0.001$). Children, especially the youngest participants, found the 5-point facial scale easier to use than the VAS. As both scales provided similar results in this study, only the results from the 5-point facial scale are presented hereafter.

Table 3
Demographics of the assessors in the paediatric and adult panels.

	Naive Paediatric Panel	Naive Adult Panel	Expert Adult Panel
n	439	65	26
Age			
Mean (years)	7.8	20.3	22
Range (years)	4—11	18—25	20—25
Paediatric Age Cohorts			
4 – 8 years (n =)	248	–	–
9 – 11 years (n =)	191	-	-
Sex			
Female (%)	47.5	59.1	69.2
Male (%)	52.5	40.9	30.8

3.3. Bitterness aversion of QHCl taste strips in paediatric and Naïve adult assessors

Bitterness aversion ratings increased in both children and adults as the quantity of QHCl on the tTS increased (Fig. 3). Overall, the bitterness aversion ratings in children were higher for all TS concentrations, demonstrating that children perceived the bitter taste of QHCl as more aversive than the young adult participants. Over twice as many children (52.6%) rated the highest concentration strip (TS4) with the extreme "It's horrible" (5) score versus (19.7%) of adults.

For all paediatric assessors, a non-parametric Friedman test showed a statistically significant difference in the responses to the different TS, $\chi2(3) = 477.456$, $p < 0.0001$. Further, a Nemenyi's post-hoc test for pairwise comparison showed that the paediatric assessors perceived a distinct and significant difference in bitterness aversion between all four TS concentrations (Fig. 4). Multiple pairwise comparisons using the Nemenyi's procedure classified the TS into individual groups (A, B, C, D) based on any significant differences in the mean of ranks.

Friedman's test also showed that bitterness aversion ratings were significantly different in the young adult assessors, $\chi2(3) = 82.732$, $p < 0.0001$. However, post-hoc multiple pairwise comparisons using the Nemenyi's procedure classified the four TS into only three groups, indicating that the most concentrated strip (TS4) (mean of ranks = 3.57) was perceived differently from all other strips; however, there was no significant difference in bitterness aversion between the lowest concentrations: TS1 (1.77) vs. TS2 (2.02) and TS2 (2.02) vs. TS3 (2.64).

Further analysis of the results from paediatric assessors identified age-related differences in responses. Bitterness aversion ratings were compared for each individual age group and similarities were seen in paediatric assessors aged 4 to 8 years and 9 to 11 years. As shown in Fig. 4, the 4 to 8 year-old cohort perceived a clear difference between the

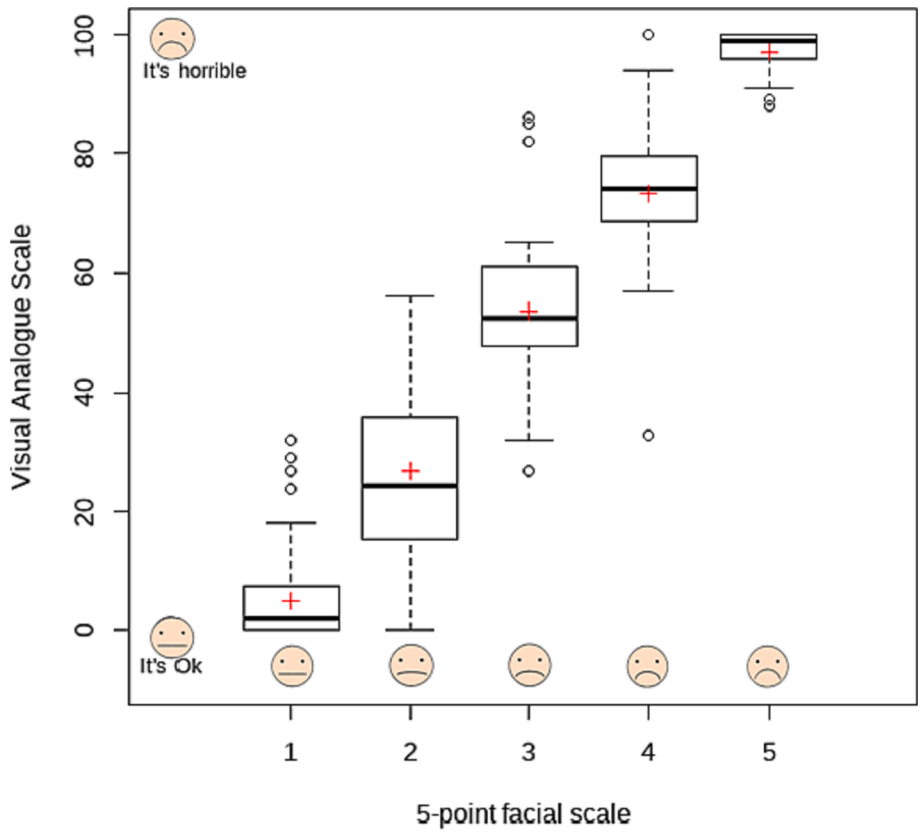

Fig. 2. Correlation between responses from paediatric assessors for all taste strips evaluations captured on the 5-point facial scale and continuous VAS.

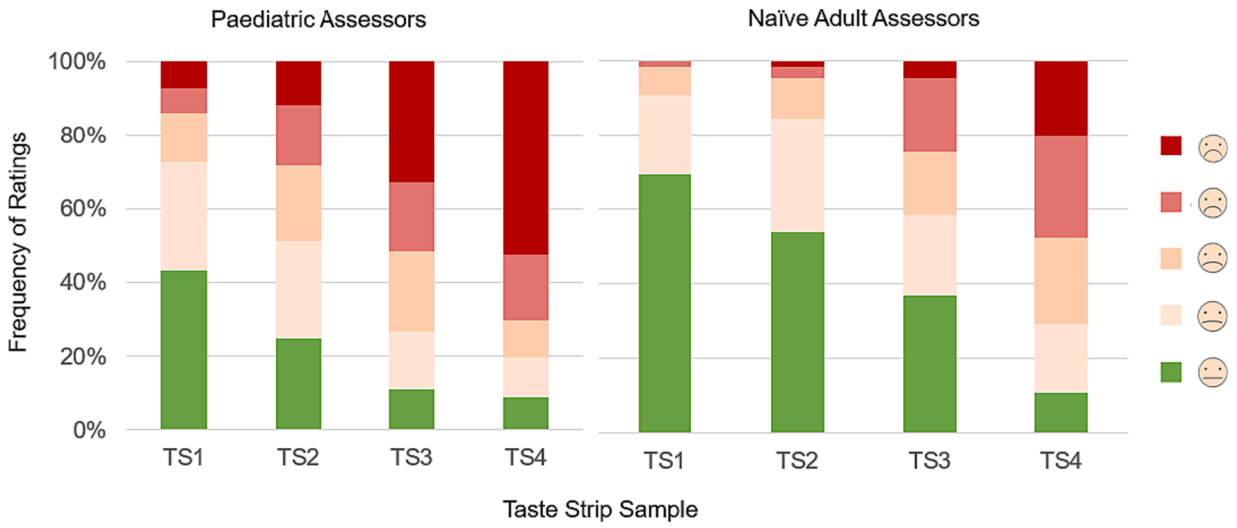

Fig. 3. Bitterness aversion ratings for four QHCl taste strips (TS1 – TS4) by a) paediatric assessors and b) naïve adult assessors captured on a 5-point facial scale.

first three TS concentrations, whereas no discrimination was observed between TS3 (mean of ranks = 3.63) and TS4 (mean of ranks = 3.85). Conversely, the 9 to 11 year-old subgroup showed no discrimination between the two lowest TS concentrations, but exhibited significant differences in bitterness aversion as the concentration of quinine increased. This trend was similar to that observed in adults, where significant discrimination in taste perception was observed at the highest TS concentration but not the lowest.

A Mann-Whitney *U* test was completed to determine if there were differences in ratings based on sex for each TS. In paediatric assessors, there was a small difference observed only for TS3, where female assessors rated the taste strip as more aversive compared to males ($p = 0.047$). In adults, a similar trend was seen where females rated two of the concentrations as more aversive compared to males. For TS2, the mean of ranks for females was 1.91 versus 1.36 for males ($p = 0.011$) and for TS4, the mean of ranks for females was 3.60 versus 2.86 for males ($p = 0.024$). Paired comparisons for all other taste strips concentrations were not statistically significantly different based on sex.

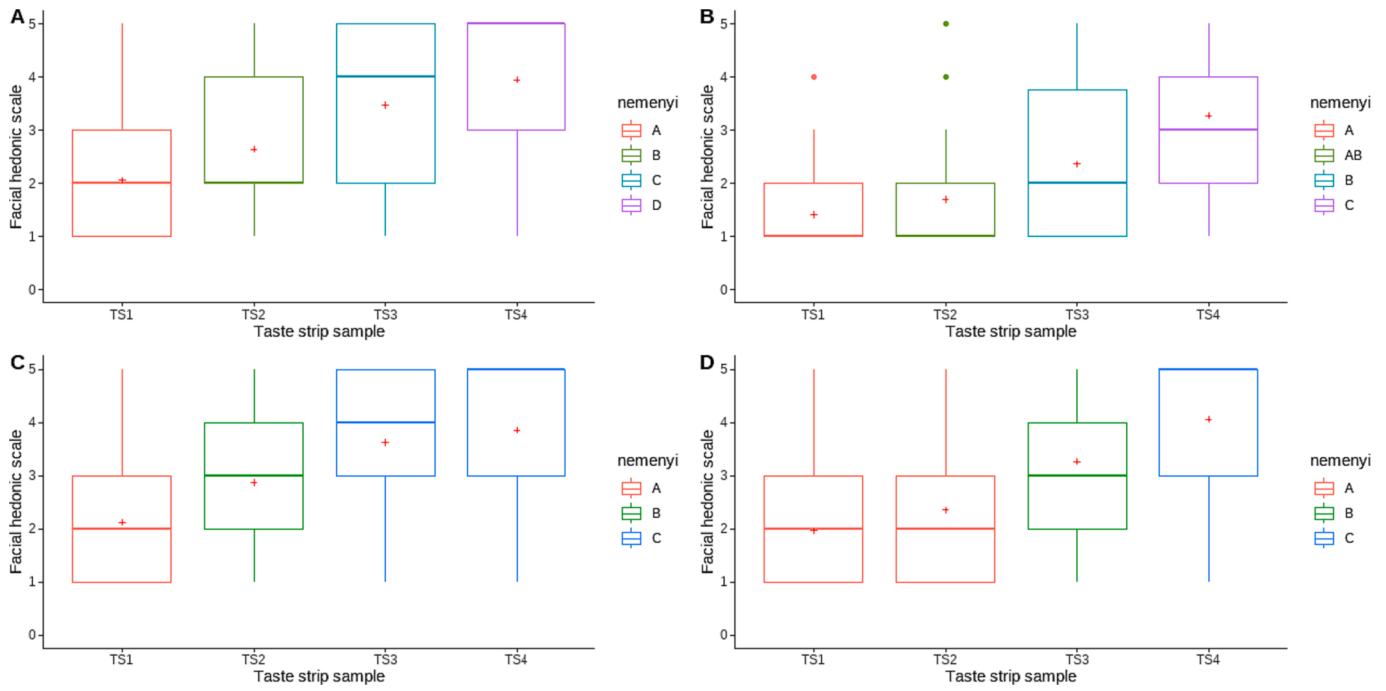

Fig. 4. Boxplots illustrating the bitterness aversion ratings for the four QHCl strips (TS1 – TS4) by (A) 4–11 year old paediatric assessors and (B) naïve adult sensory assessors. Ratings for paediatric subgroups are also presented: (C) 4–8 years and (D) 9–11 years. Coloured boxes A-D in the legends highlight groupings following Nemenyi's post-hoc test.

3.4. Bitterness aversion of QHCl taste strips (TS) vs. Aqueous solutions (AS) in Naïve and expert adult assessors

The naïve and expert young adult assessors evaluated QHCl solutions theorised to be at equivalent concentrations to the TS. The concentrations of the AS were established according to precedent studies (Georgopoulos, Keeley et al. 2019) showing that the QHCl in water needed to be diluted with 3 times to present the same amount of drug in the mouth as the taste strips. However, in this study, the bitterness aversion ratings for both mediums were found to be offset as shown in Figs. 5 and 6.

The evaluations of the naïve panellists using the 5-point hedonic scale allowed discrimination between the AS samples, but for the TS, the panellists did not discriminate the lowest quantities. Thus, for a medium or high concentration or more biter compounds TS are a reliable option. This confirms the previous observations.

When expert panellists rated bitterness on a discrete scale [0 to 10] (Fig. 6), the same trends were noted. The averages are more biased in TS than AS, and the discriminating capacity is stronger with solution than for strips. It is, however, interesting to note that for the strips TS1, TS3 and TS4 are significantly different. Interestingly, expert panellists perceived all samples as less bitter than naïve adult panellists perceived their aversiveness.

4. Discussion

This study compared how school-aged children and young adults perceived the dose dependent aversiveness of the same bitter stimuli, namely four TS impregnated with different concentrations of QHCl. The TS proved to be a safe and easy OOL methodology, allowing taste evaluation to be conducted rapidly in a large number of children as young as 4 years old. Analysis by age showed a similar trend among children aged 4 to 8 years, who discriminated the lower TS concentrations but did not perceive a significant difference in aversiveness between TS3 and TS4. Conversely, children aged 9 to 11 years showed responses similar to the young adults, where discrimination was seen for the higher strip concentrations, but not between TS1 and TS2.

Taste perception is a complex and multifaceted phenomenon, and individual differences in sensitivity and response can be influenced by numerous physiological and environmental factors including age, sex, genetics, ethnicity, nutritional status, health status, and exposure to different foods (including in utero), to name a few (Beckett et al., 2014, Nehring et al., 2015, Shizukuda et al., 2018, Diószegi et al., 2019). Bitter taste perception in humans is primarily mediated by TAS2Rs, a family of are G-protein-coupled receptors expressed on the surface of taste buds (Wooding et al., 2021). Compounds such as QHCl act as agonists for these receptors and trigger a series of biochemical signalling cascades and neural activity resulting in bitterness perception. There are 25 subtypes of TAS2Rs, each recognising multiple bitter substances, while a single bitter compound can activate several TAS2Rs. Quinine has shown to activate different receptors in the micromolar range (Meyerhof et al., 2010), and sensitivity to its taste intensity has been linked to *TAS2R19* genotype (Reed et al., 2010, Deng et al., 2022).

The aim of this study was to better understand how taste perception data from adults can be used to support the development of medicines for children, therefore age was the main variable investigated. Overall, the paediatric assessors rated the TS as more aversive compared to the young adults, which is in line with the findings of a review highlighting that children are more sensitive to bitter tastes (Mennella, Spector et al. 2013). Mennella et al. showed that children aged 5 to 10 years (mean 8.0 ± 0.2 years) were more sensitive to the bitterness of propylthiouracil (PROP) compared to adults with the same taste-sensitive *TAS2R38* genotype (mean age 35.3 ± 0.6 years and mothers of the child participants), highlighting that the impact of age is independent of genetics (Mennella et al., 2005). A later study showed that children aged 3 to 10 years old showed increased bitterness sensitivity, whereas from adolescence (11 to 19 years), the relationship between receptor genotype and bitterness perception started to resemble the adult pattern (Mennella, Pepino et al. 2010). In the present study, the slightly younger "pre-adolescent" cohort aged 9–11 years showed similar trends to the young adults. A longitudinal study with Norwegian school children showed that sensitivity to the bitter taste of QHCl remained stable in children from 4 to 6 years, while other taste sensitivities changed over

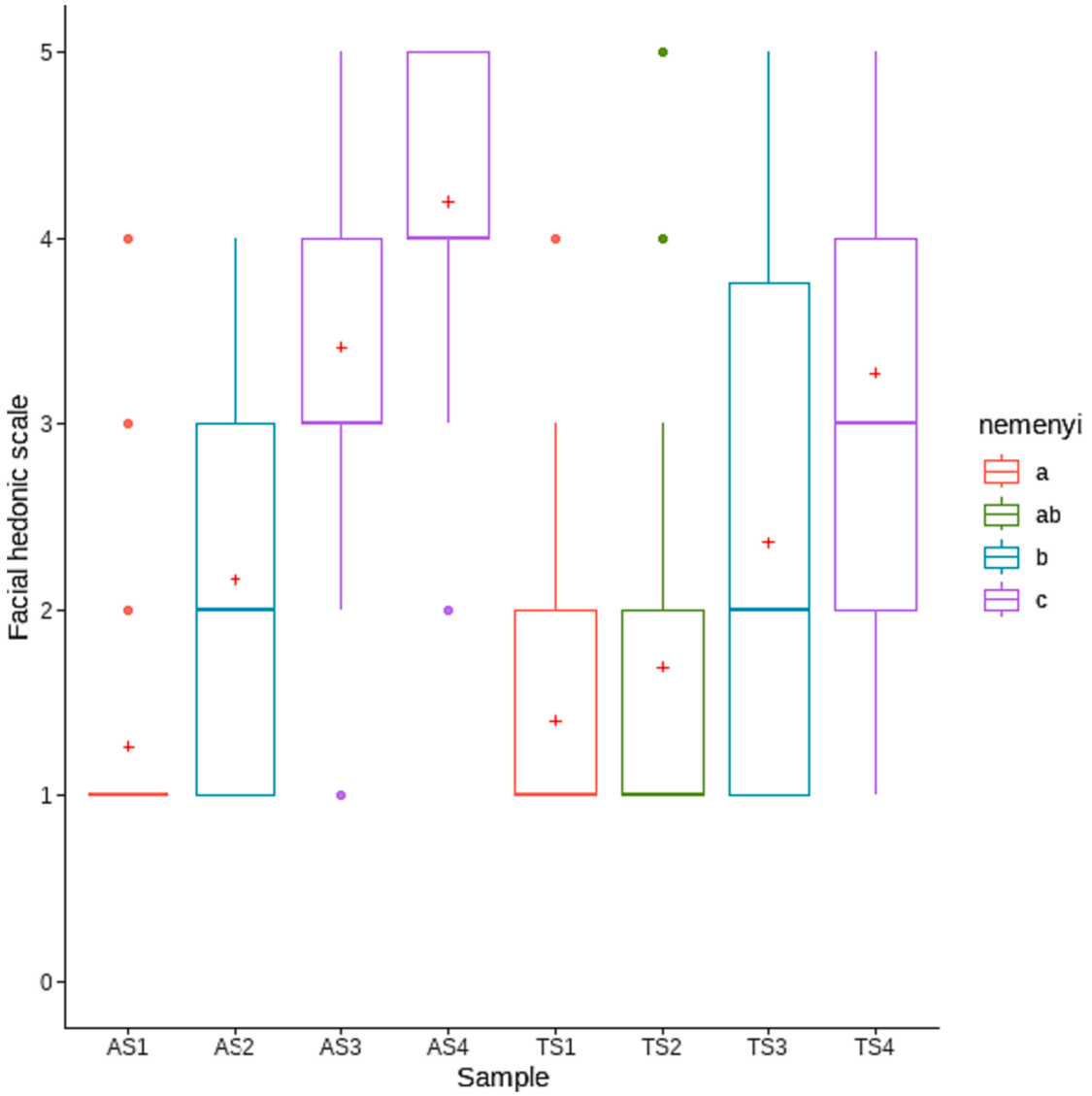

Fig. 5. Boxplot illustrating the bitterness aversion ratings for the QHCl solutions (AS1 – AS4) and strips (TS1 – TS4) as evaluated by the naïve panel on the 5-point facial scale. (ab means that the product is not significantly different from products in group a either in group b).

the same time (e.g. sensitivity to saltiness and sourness increased while sweetness decreased) (Fry Vennerød, Nicklaus et al. 2018). Similarly, no significant differences in responses were seen among the youngest participants in the study.

While the aforementioned studies evaluated taste detection thresholds (a minimum concentration at which the taste could be detected) using solution samples, the present study investigated hedonic responses to bitter stimuli with TS. Most other studies using TS in children have aimed to measure taste function by examining participants' ability to recognise the specific taste quality of the strip rather than perceived intensity or degree of liking. Recently, however, Schienle et al. captured intensity of taste sensation (from no sensation to "the strongest imaginable sensation of any kind") also using QHCl TS. Overall, the authors observed no difference in ratings between children aged 7 to 11 years (mean 8.5 ± 1.0 years) and adults (mean 29.42 ± 11.37 years) (Schienle 2020). However, participants only evaluated a single bitter TS impregnated with 0.006 g/mL QHCl. There were also other methodological differences which may have contributed to the difference in findings; for example, the use of a glms scale, the time of TS contact on the tongue was uncertain and the tongue may have remained extended while testing the strip compared to the closed mouth 10 s testing protocol implemented in the present study.

Physiological differences can contribute to the differences observed between children and adults. A study investigating tongue development in subjects aged 4 to 32 years found that the area of the anterior region of the tongue reached adult size by 8 to 10 years, while the posterior region continue to grow until 15–16 years (Temple et al., 2002). The anterior region of the tongue is rich in fungiform papillae, the raised structures on the tongue that house taste buds. Correa et al. examined fungiform papillae density as a function of age and showed that the children 7–8 years old (the youngest study participants) has around 13.6% more papillae compared to adults, which was a statistically significant difference (Correa, Hutchinson et al. 2013). Moreover, the number of papillae decreased with age before stabilising at around 9 to 10 years of age, whereas their size, shape and distribution stabilised by 11 to 12 years (Correa, Hutchinson et al. 2013). Collectively, these findings are in line with the age-related differences observed in this study.

Sex was investigated as a secondary outcome and its impact was less clear. At some TS concentrations, female children and adults rated the samples as more aversive than males, but this was not conclusive across all paired comparisons. Schienle et al. found that women rated the taste intensity of the QHCl strip higher than men, but the same sex-related

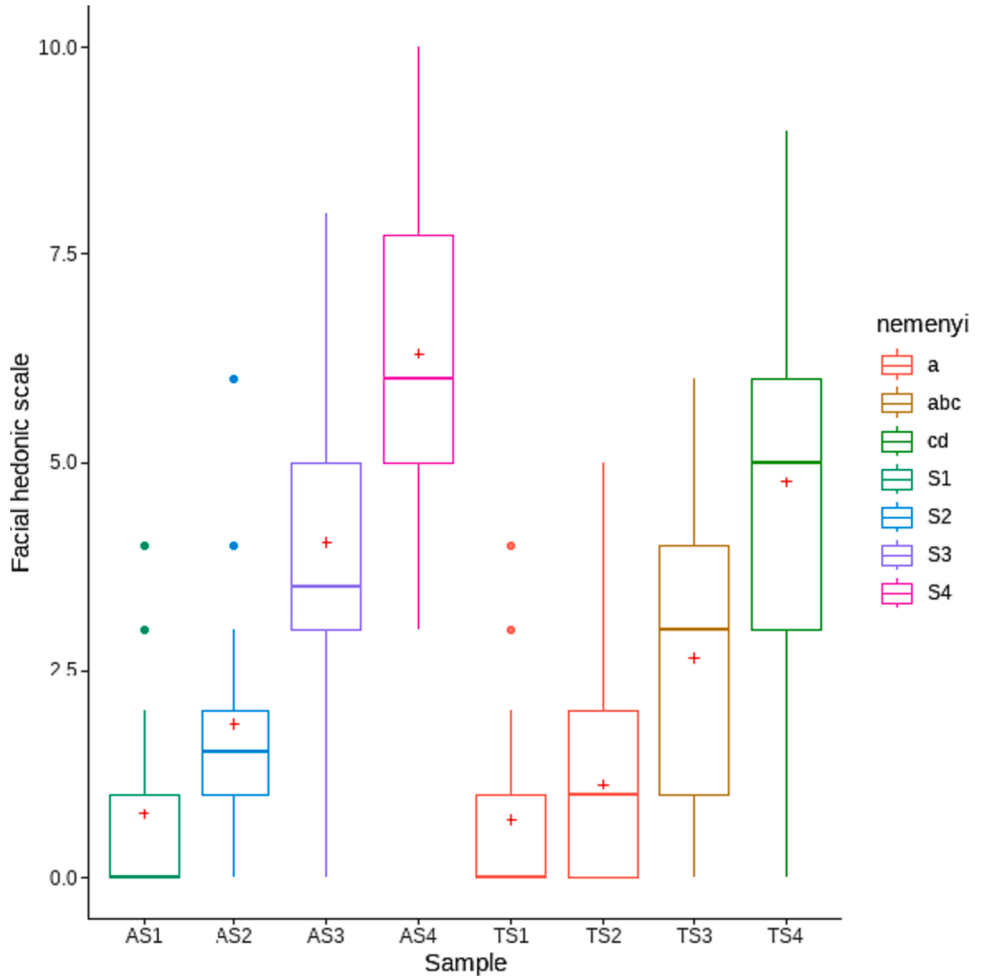

Fig. 6. Boxplot illustrating the bitterness intensity ratings for the QHCl solutions (AS1 – AS4) and strips (TS1 – TS4) as evaluated by the expert panel on an 11-point discrete numerical scale.

effect was not seen in children (Schienle 2020). Mennella et al. found no sex-related differences in PROP thresholds in children and adolescents (Mennella, Pepino et al. 2010). There were also no sex-related differences seen for tongue dimensions or fungiform papillae density (Temple et al., 2002, Correa et al., 2013).

Cultural differences and related dietary habits can influence sensory perception and preferences; however, empirical data on this are limited. Current standards on sensory evaluation do not provide guidance on this aspect when selecting sensory assessors. Although participant's ethnicity was not captured in this study, cross-cultural sampling would be advantageous in future studies.

The present study was unique in capturing hedonic responses in the naïve children and adult assessors, namely the degree to which the TS was liked or disliked. Ervina et al., 2020 conducted an in-depth study investigating taste perception in 11-year old children using 10 mL solutions of both quinine and caffeine as bitter tastants (Ervina et al., 2020). Interestingly, hedonic taste liking, captured on seven-point pictorial scale including a happy and grim face, did not correlate with sensitivity measures including detection thresholds and recognition thresholds (Ervina et al., 2020). It is therefore important that affective responses and palatability data are captured independently of determining taste detection thresholds or measuring the intensity of bitter tasting APIs.

In an era of patient-centric drug development, there is an important need to develop tools to safely and accurately gauge taste perception in children themselves, as well as with adults. Alongside ethical and toxicological concerns, there are additional complexities related to conducting pharmaceutical sensory studies especially in the paediatric population. This includes participant burden, e.g. the number of samples that can be evaluated to minimise fatigue and cater to shorter attention spans; cognitive abilities e.g. following test instructions and using age-adapted scales; risks related to inadvertent swallowing; and difficulty if ability at all, for some very young children to verbalize the aversiveness (Johnson et al., 2021). The TS methodology offers a quick, safe, and accurate method to assess bitterness perception, and both the VAS and hedonic scale were successfully used with young children.

Further work is also needed to better understand how TS evaluations could be correlated with more traditional whole mouth testing using the "swirl and spit" technique. The concentrations of TS and AS tested here with naïve and expert adult panellists were not comparable, contrary to the results of a prior proof of concept study (Georgopoulos, Keeley et al. 2019). Average scores were systematically higher with the AS than TS for all adults, and the range of aversiveness ratings was wider for AS. There were also differences observed between the expert and naïve panels. The expert panel rated the bitterness intensity as low or medium, when it was still aversive to the naïve assessors. It is possible that the expert assessors may have become habituated to bitter tastes due to the repetition of the sensory evaluations during their training.

Drug loading on the TS was not optimal, thus, there was some loss of discrimination. Moreover, the differences could also be explained by the fact that swirling 10 mL AS around the mouth exposes more receptors to the taste stimuli, whereas localised strip assessment on the tongue is more prone to variability in taste bud exposure. Another parallel consideration is the residual salivary volume in the mouth. A recent

extensive literature review reported that the salivary flow rate can be influenced by numerous factors including age. Basal and stimulated salivary flow rates were reported to vary from 0.18 to 2.29 mL/min in school children compared to 0.19 to 7 mL/min in adults (Wollmer, Ungell et al. 2022).

Future work should focus on exploring TS loading in relation with the level of API aversiveness and its maximum solubility. In addition, this study used QHCl, a highly soluble compound with a well-defined bitterness profile, therefore further work is needed to evaluate APIs that elicit a range of bitterness intensities and taste sensations to determine if the methodology is more universally applicable. Despite, the observed difference in sensitivity to bitterness, the adult panel remains the best alternative to evaluate the taste of drugs, for ethical reasons and because of their ability to describe samples. Approaches to select more sensitive naïve adult assessors who can discriminate between low level bitter stimuli should be explored, to be closer to the younger children who have a heightened bitter sensitivity.

5. Conclusion

Medicines for children needs to be palatable enough not to hinder acceptability and compliance from the product point of view. APIs often taste bitter especially to children. However, to inform paediatric drug development, healthy adult volunteers are typically enrolled for preclinical pharmaceutical sensory research studies although methodological questions related to the level of expertise required (expert or naïve) from these assessors remains unclear.

Presently, children and adults were able to differentiate the degree of aversiveness of each TS concentration. Data from the children's panel could be split into two groups, 4–8 years and 9–11 years, based on the age-related variation in responses that was observed. Younger children demonstrated a discrimination threshold in the two lower TS concentrations but were not able to discriminate the two highest concentrations. Conversely, children 9–11 years old and naïve adults showed better discrimination of the higher TS concentrations. Across all TS concentrations, children rated the samples more aversive than the adult panellists. Expert panellist perceived all the samples less bitter compared to naïve panellist for strips and for aqueous solutions. The expert panel rated the bitterness intensity as low or medium, whereas it was still aversive to the naïve panel which in turn could be more representative of children with heightened bitter aversion. Therefore, resourcing to adults is logical for safety and ethical reasons but with appropriate study design, they can play an important role in providing valuable data that can aid and guide paediatric formulation development. Further studies are required to establish a correlation between children and adult panels for aversiveness assessment with a wider spectrum of bitter tastants.

CRediT authorship contribution statement

Sejal R Ranmal: Data curation, Formal analysis, Visualization, Writing – original draft. **Zeineb Nhouchi:** Data curation, Formal analysis, Writing – review & editing. **Alexander Keeley:** Data curation, Writing – review & editing. **Lisa Adler:** Data curation, Visualization, Writing – original draft. **Marc Lavarde:** Formal analysis, Methodology, Visualization. **Anne-Marie Pensé-Lhéritier:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Catherine Tuleu:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

AAP Division of Health Policy Research (2001). "Many patients don't comply with prescription regimens: survey." AAP News **18**(5): 213–213.

Al-Kasmi, B., Al Rahal, O., El-Zein, H., Nattouf, A.H., 2018. Structural and in vitro in vivo evaluation for taste masking. Expert Opin. Drug Deliv. 15 (11), 1105–1116.

Baguley, D., Lim, E., Bevan, A., Pallet, A., Faust, S.N., 2012. Prescribing for children - taste and palatability affect adherence to antibiotics: a review. Arch. Dis. Child. 97 (3), 293–297.

Banek, K., DiLiberto, D.D., Webb, E.L., Smith, S.J., Chandramohan, D., Staedke, S.G., 2021. Exploring Barriers and Facilitators of Adherence to Artemisinin-Based Combination Therapies for the Treatment of Uncomplicated Malaria in Children in Freetown, Sierra Leone. Healthcare (Basel) 9 (9).

Beckett, E.L., Martin, C., Yates, Z., Veysey, M., Duesing, K., Lucock, M., 2014. Bitter taste genetics–the relationship to tasting, liking, consumption and health. Food Funct. 5 (12), 3040–3054.

Breslin, P.A., 2013. An evolutionary perspective on food and human taste. Curr. Biol. 23 (9), R409–R418.

Chambers, C.T., Craig, K.D., 1998. An intrusive impact of anchors in children's faces pain scales. Pain 78 (1), 27–37.

Clapham, D., Bennett, J., Cram, A., Discihnger, A., Inghelbrecht, S., Pense-Lheriter, A.M., Ruiz, F., Salunke, S., Schiele, J., Soto, J., Walsh, J., Tuleu, C., 2021. Proposed Tool to Compare and Assess the Applicability of Taste Assessment Techniques for Pharmaceuticals. J. Pharm. Sci.

Correa, M., Hutchinson, I., Laing, D.G., Jinks, A.L., 2013. Changes in fungiform papillae density during development in humans. Chem. Senses 38 (6), 519–527.

Deng, M., Hida, N., Yamazaki, T., Morishima, R., Kato, Y., Fujita, Y., Nakamura, A., Harada, T., 2022. Comparison of Bitterness Intensity between Prednisolone and Quinine in a Human Sensory Test Indicated Individual Differences in Bitter-Taste Perception. Pharmaceutics 14 (11).

Diószegi, J., Llanaj, E., Ádány, R., 2019. Genetic Background of Taste Perception, Taste Preferences, and Its Nutritional Implications: A Systematic Review. Front. Genet. 10, 1272.

Ervina, E., Berget, I., Almli, V.L., 2020. Investigating the Relationships between Basic Tastes Sensitivities, Fattiness Sensitivity, and Food Liking in 11-Year-Old Children. Foods 9 (9).

European Medicines Agency (2013). Guideline on pharmaceutical development of medicines for paediatric use (EMA/CHMP/QWP/805880/2012 Rev. 2).

Fry Vennerød, F.F., Nicklaus, S., Lien, N., Almli, V.L., 2018. The development of basic taste sensitivity and preferences in children. Appetite 127, 130–137.

Georgopoulos, D., A. Keeley and C. Tuleu (2019). Assessing the feasibility of using "taste-strips" for bitterness taste panels. 11th European Paediatric Formulation Initiative (EuPFI) Conference, Malmo, Sweden.

Guedes, M.D.V., Marques, M.S., Guedes, P.C., Contri, R.V., Kulkamp Guerreiro, I.C., 2021. The use of electronic tongue and sensory panel on taste evaluation of pediatric medicines: a systematic review. Pharm. Dev. Technol. 26 (2), 119–137.

Gutierrez-Colina, A.M., Smith, A.W., Mara, C.A., Modi, A.C., 2018. Adherence barriers in pediatric epilepsy: From toddlers to young adults. Epilepsy Behav. 80, 229–234.

Hill, C.A., Beach, M., Smith, M.C., Chen, E.Y., 2016. Incidence of and Factors Associated With Hypogeusia in Healthy Children. JAMA Otolaryngol. Head Neck Surg. 142 (3), 229–233.

International Organization for Standardization (2003). Sensory analysis — Guidelines for the use of quantitative response scales (ISO 4121:2003).

International Organization for Standardization (2012). Sensory analysis — General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors (ISO 8586:2012).

International Organization for Standardization (2016). Sensory Analysis - Vocabulary - Amendment 1 (ISO 5492:2008/AMD 1:2016).

Johnson, S.L., Moding, K.J., Grimm, K.J., Flesher, A.E., Bakke, A.J., Hayes, J.E., 2021. Infant and Toddler Responses to Bitter-Tasting Novel Vegetables: Findings from the Good Tastes Study. J. Nutr. 151 (10), 3240–3252.

Landis, B.N., Welge-Luessen, A., Brämerson, A., Bende, M., Mueller, C.A., Nordin, S., Hummel, T., 2009. "Taste Strips" - a rapid, lateralized, gustatory bedside identification test based on impregnated filter papers. J. Neurol. 256 (2), 242–248.

Mennella, J.A., Bobowski, N.K., 2015. The sweetness and bitterness of childhood: Insights from basic research on taste preferences. Physiol. Behav. 152 (Pt B), 502–507.
Mennella, J.A., Pepino, M.Y., Reed, D.R., 2005. Genetic and environmental determinants of bitter perception and sweet preferences. Pediatrics 115 (2), e216–e222.
Mennella, J.A., Pepino, M.Y., Duke, F.F., Reed, D.R., 2010. Age modifies the genotype-phenotype relationship for the bitter receptor TAS2R38. BMC Genet. 11, 60.
Mennella, J.A., Spector, A.C., Reed, D.R., Coldwell, S.E., 2013. The bad taste of medicines: overview of basic research on bitter taste. Clin. Ther. 35 (8), 1225–1246.
Meyerhof, W., Batram, C., Kuhn, C., Brockhoff, A., Chudoba, E., Bufe, B., Appendino, G., Behrens, M., 2010. The molecular receptive ranges of human TAS2R bitter taste receptors. Chem. Senses 35 (2), 157–170.
Mohamed-Ahmed, A.H., Soto, J., Ernest, T., Tuleu, C., 2016. Non-human tools for the evaluation of bitter taste in the design and development of medicines: a systematic review. Drug Discov. Today 21 (7), 1170–1180.
Mueller, C., Kallert, S., Renner, B., Stiassny, K., Temmel, A.F., Hummel, T., Kobal, G., 2003. Quantitative assessment of gustatory function in a clinical context using impregnated "taste strips". Rhinology 41 (1), 2–6.
Nehring, I., Kostka, T., von Kries, R., Rehfuess, E.A., 2015. Impacts of in utero and early infant taste experiences on later taste acceptance: a systematic review. J. Nutr. 145 (6), 1271–1279.
Nordenmalm, S., Kimland, E., Ligas, F., Lehmann, B., Claverol, J., Nafria, B., Tötterman, A.M., Pelle, B., 2019. Children's views on taking medicines and participating in clinical trials. Arch. Dis. Child. 104 (9), 900–905.
Overberg, J., Hummel, T., Krude, H., Wiegand, S., 2012. Differences in taste sensitivity between obese and non-obese children and adolescents. Arch. Dis. Child. 97 (12), 1048–1052.
Reed, D.R., Zhu, G., Breslin, P.A., Duke, F.F., Henders, A.K., Campbell, M.J., Montgomery, G.W., Medland, S.E., Martin, N.G., Wright, M.J., 2010. The perception of quinine taste intensity is associated with common genetic variants in a bitter receptor cluster on chromosome 12. Hum. Mol. Genet. 19 (21), 4278–4285.
Sauer, H., Ohla, K., Dammann, D., Teufel, M., Zipfel, S., Enck, P., Mack, I., 2017. Changes in Gustatory Function and Taste Preference Following Weight Loss. J. Pediatr. 182, 120–126.
Schienle, A., Schlintl, C. (2020). " The Association Between Quinine Hydrochloride Sensitivity and Disgust Proneness in Children and Adults." Chem. Percept.**13**: 78–83.
Shizukuda, S., Marchini, J.S., Adell, A., Santos, M.A., Brandao, C.F.C., Lima, C.M.M., Cunha, S.F.C., Itikawa, E.N., Silvah, J.H., 2018. Influences of weight, age, gender, genetics, diseases, and ethnicity on bitterness perception: a narrative review of current methodological aspects. Nutrire 43 (1), 4.
Temple, E.C., Hutchinson, I., Laing, D.G., Jinks, A.L., 2002. Taste development: differential growth rates of tongue regions in humans. Brain Res. Dev. Brain Res. 135 (1–2), 65–70.
van den Brink, M., I. IJpma, M. Fiocco, W. J. E. Tissing and R. C. Havermans (2021). "Taste function in children: normative values and associated factors." Pediatr Res.
Venables, R., Batchelor, H., Hodson, J., Stirling, H., Marriott, J., 2015. Determination of formulation factors that affect oral medicines acceptability in a domiciliary paediatric population. Int. J. Pharm. 480 (1–2), 55–62.
Wollmer, E., Ungell, A.L., Nicolas, J.M., Klein, S., 2022. Review of paediatric gastrointestinal physiology relevant to the absorption of orally administered medicines. Adv. Drug Deliv. Rev. 181, 114084.
Wooding, S.P., Ramirez, V.A., Behrens, M., 2021. Bitter taste receptors: Genes, evolution and health. Evol Med Public Health 9 (1), 431–447.