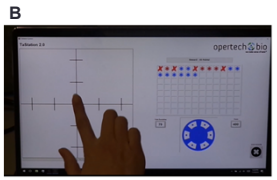
High Throughput Characterization of Aloin Bitter Taste Phenotypes in Human Subjects

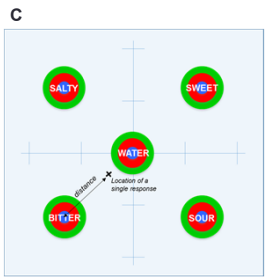
Mariah Stewart, and R. Kyle Palmer
Opertech Bio, Inc., Philadelphia, PA, United States

ABSTRACT

We previously reported a new technology, called the TaStation®, for high throughput generation of concentration-response (CR) functions for bitter taste using human subjects (The FASEB Journal, 31 (1) Suppl 1059.4). The method is based on an operant taste discrimination paradigm in which small volume (200 ul) samples of tastant solutions are randomly selected and robotically drawn from a 96-well plate, then presented to a subject who is trained to distinguish among basic taste stimuli with high acuity and consistency. The 8x12 matrix of the 96-well plate is a convenient format for arranging tastants in concentration ranges with multiple replicates, and a subject can sample all 96 wells within a 40 minute test session. Thus, robust CR functions for tastants can be established quickly to yield precise EC50 values for single subjects. We now report the use of the TaStation® for characterizing taste phenotypes for individual human subjects. Aloin, a bitter substance isolated from Aloe sap, has been shown to be a specific agonist of the TAS2R43, and human taste sensitivity to aloin is dependent upon the allele of this receptor expressed by the individual (Current Biology, 17, 1403.) Eight concentrations of aloin and two other bitter tastants, quinine and the antihistamine drug diphenhydramine, were dispensed in triplicate into single 96-well plates and presented to 4 subjects (2 male, 2 female) trained to use the TaStation® for bitter detection. Also distributed among the remaining 24 wells were stimuli representing the basic tastes (0.5 mM quinine—bitter; 100 mM NaCl—salty; 100 mM sucrose—sweet; 10 mM citric acid—sour), and water. The test consisted of discriminating the sample from the basic taste cues and water. After tasting a sample, subjects recorded their response on a touch-sensitive computer display by touching invisible target locations they previously had been trained to associate with each basic taste and water. Correct responses on control trials were rewarded with a virtual poker chip and associated point value, and if incorrect were penalized by a reduction in point value and a 30-second delay before the next trial. All responses on trials of the tastant concentration ranges were rewarded regardless of location. Each subject repeated this test twice, with each test conducted on separate days. The resulting CR functions established at least two clear phenotypes for aloin taste. Two subjects generalized the taste of aloin to the bitter cue with EC50s of 3 uM (95%CI = 2-4 uM) and 13 uM (95%CI = 11-16 uM), whereas the other two subjects appeared to be insensitive to the taste of aloin, generalizing to the water cue even at the highest concentration of 100 uM. In contrast, EC50s for quinine ranged between 51 and 182 uM among the four subjects, and diphenhydramine EC50s for all four subjects were essentially equivalent at ~1 mM. Subject genotyping will be carried out to determine TAS2R43 alleles.

AUTOMATED SYSTEM FOR HIGH THROUGHPUT MEASUREMENT OF HUMAN TASTE 1



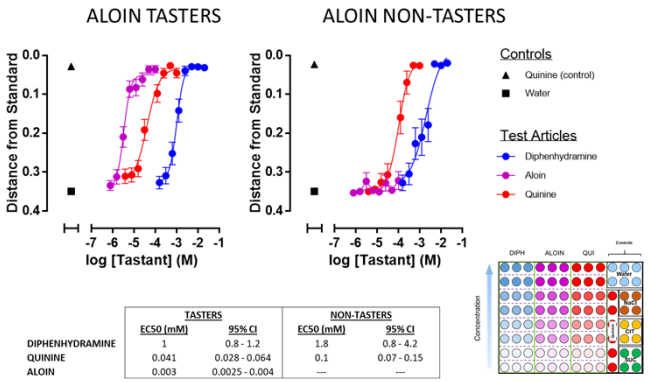


A) Robotic gantry moves an automated pipette over a 96-well plate. The pipette is lowered into a randomly selected well and withdraws a fixed volume of 200 ul. The subject is instructed by the algorithm to remove the pipette and self-administer the content of the pipette to the tongue.

B) Subjects search for poker chips buried in a visual field; the taste stimulus is a clue to their location. The subject touches the screen at a location guided by the taste of the antecedent stimulus. Response-reinforcement contingency is absolute on control trials (taste standards). On test article trials—those for novel stimuli—all responses are reinforced.

C) The distance between the coordinates of the subject's response and the ideal coordinates of the target is measured and recorded as the datum.

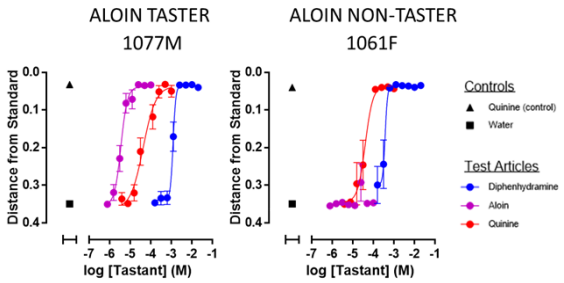
CONCENTRATION-RESPONSE FUNCTIONS FOR BITTER TASTANTS: TASTERS VS. NON-TASTERS 2



	TASTERS		NON-TASTERS	
	EC50 (mM)	95% CI	EC50 (mM)	95% CI
DIPHENHYDRAMINE	1	0.8 - 1.2	1.8	0.8 - 4.2
QUININE	0.041	0.028 - 0.064	0.1	0.07 - 0.15
ALOIN	0.003	0.0025 - 0.004	---	---

Concentration-response functions for bitter taste stimuli. ***Group Data:*** Cohort is composed of 2 male and 3 female adult subjects tested with a 96-well plate (see inset lower right) in which concentration ranges of three bitter tastants was dispensed. Data are plotted as distance of each subject's response from the x-y coordinates assigned to the quinine standard. Each data point in the curves was calculated as the average across 30 replicates (3 replicates per concentration x 2 tests x 5 subjects). The results indicate robust concentration-response data across a vast range of potencies among these bitter tastants. ***Concentration-response Functions and EC50s:*** Curves were fit to the data points by non-linear regression to generate concentration-response function for each of the tastants. EC50s were derived from the curve fits.

INDIVIDUAL SUBJECT DATA 3

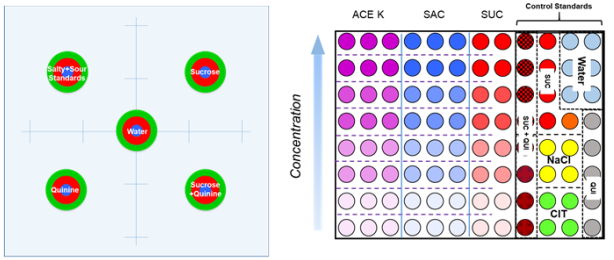


Bitter taste concentration-response functions for individual taster and non-taster subjects. Data are plotted as described above for one aloin taster and one non-taster. Each data point represents the average of 6 replicates (3 replicates x 2 tests).

INDIVIDUAL SUBJECT RESULTS ARE CONSISTENT ACROSS TEST SESSIONS 4

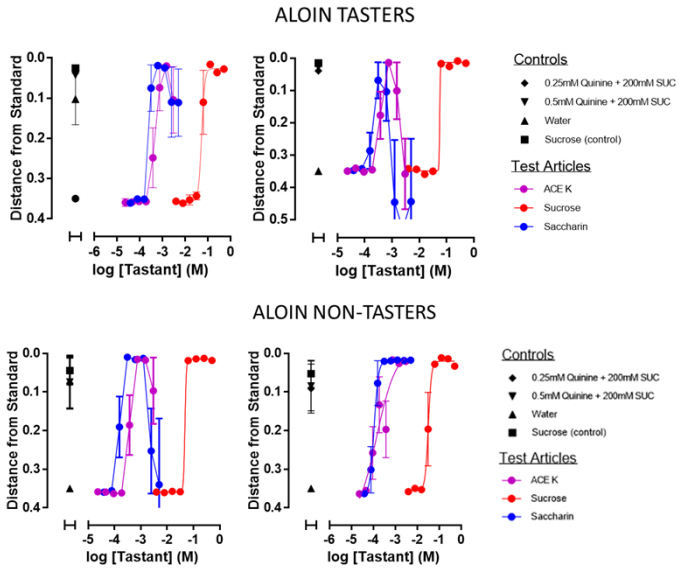
	SUBJECT 1077 M					
Test Date	12/5/2017	12/12/2017	3/10/2018	3/12/2018	3/13/2018	3/14/2018
	EC50 (mM)					
DIPHENHYDRAMINE	1.2	1.4	1.2	1.3	1.3	0.71
QUININE	0.05	0.06	0.03	0.11	0.05	0.07
ALOIN	0.003	0.006	0.003	0.014	0.003	0.004

PROCEDURE FOR TRAINING SENSITIVITY TO BITTER OFF-TASTES IN A BACKGROUND OF SWEET TASTE 5



LEFT PANEL: Subjects were trained to discriminate pure sweet from bittersweet, pure bitter, water, salty, and sour taste standards by associating their tastes with specific coordinates on a touch sensitive display (targets were invisible to the subject). **RIGHT PANEL:** In test sessions, subjects' responses were recorded on targets associated with pure sweet, bittersweet, and pure bitter as a function of concentration. Control standards were also included in every test session.

TASTER STATUS WAS NOT AN ABSOLUTE DETERMINANT OF BITTER OFF-TASTE RECOGNITION 6



- **Some aloin non-tasters detect bitter off-tastes of Ace K and saccharin.** The data shown are from individual subjects, one male and one female each from aloin taster and non-taster groups, each tested twice.
- **NEXT STEP:** Test for phenotypes of sensitivity to aristolochic acid, which is potently selective for both TAS2R43 and the closely related TAS2R44(31) receptors.
- **CONCLUSION:** Aloin is a potent, highly selective agonist for TAS2R43, which has been shown to be responsive to saccharin and acesulfame potassium (ACE K), as well as quinine. By using a set of selective agonists of TAS2R receptors (such as aloin and aristolochic acid) in conjunction with the TaStation® technology, a rapid process of elimination can narrow down the set of candidate receptors responsible for the taste of more promiscuous bitter-tasting ligands (such as quinine) for any individual subject.