



# Data gaps in toxicity testing of chemicals allowed in food in the United States<sup>☆</sup>

Thomas G. Neltner<sup>a,\*</sup>, Heather M. Alger<sup>a</sup>, Jack E. Leonard<sup>b</sup>, Maricel V. Maffini<sup>a</sup>

<sup>a</sup> The Pew Charitable Trusts, 901 E Street NW, Washington, DC 20004, USA

<sup>b</sup> Environmental Management Institute, 5610 Crawfordsville Road, Indianapolis, IN 46224, USA

## ARTICLE INFO

### Article history:

Received 3 April 2013

Received in revised form 29 July 2013

Accepted 31 July 2013

Available online xxx

### Keywords:

Food chemicals, Food additives, FDA, GRAS, Safe, Toxicology, Food contact substances, Flavors

## ABSTRACT

In the United States, chemical additives cannot be used in food without an affirmative determination that their use is safe by FDA or additive manufacturer. Feeding toxicology studies designed to estimate the amount of a chemical additive that can be eaten safely provide the most relevant information. We analyze how many chemical additives allowed in human food have feeding toxicology studies in three toxicological information sources including the U.S. Food and Drug Administration's (FDA) database. Less than 38% of FDA-regulated additives have a published feeding study. For chemicals directly added to food, 21.6% have feeding studies necessary to estimate a safe level of exposure and 6.7% have reproductive or developmental toxicity data in FDA's database. A program is needed to fill these significant knowledge gaps by using *in vitro* and *in silico* methods complemented with targeted *in vivo* studies to ensure public health is protected.

© 2013 The Authors. Published by Elsevier Inc. All rights reserved.

## 1. Introduction

More than 10,000 chemicals are allowed to be added, directly or indirectly, to human food pursuant to the United States' (US) Food Additives Amendment of 1958 as administered by the U.S. Food and Drug Administration (FDA) [1]. They perform many roles, including preserving flavor, enhancing taste or appearance, preventing spoilage, and as constituents of packaging. As of 2010, over 90% of these additives were allowed in human food under the legal categories known as "food additives" or as "generally recognized as safe" (GRAS) substances in roughly equal numbers. GRAS substances range from common food ingredients (such as wheat) to newly engineered substances using biotechnology [1]. The remaining 10% are in smaller categories such as color

additives, pesticides, or substances whose use the federal government sanctioned before the law was enacted in 1958.

By law, food additives and GRAS substances (collectively referred to in this article as chemical additives) cannot be used in food without an affirmative determination that their use is safe (21 U.S.C. §321 and §348) by FDA or, in some cases, the additive manufacturer. Safety means that there is a "reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use" (21 CFR §170.30(i)). Also, "no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal" (21 U.S.C. §348(c)(3)(A)) [1]. Congress required affirmative determinations and set stringent standards for safety, acknowledging that the health effects of chemical additives are often hidden or take years to show up. These standards aim to protect the public as well as to encourage innovation and build public confidence in the safety of the food supply [2].

In addition, a safety decision for a GRAS substance based on scientific procedures must be supported by published studies (whether peer-reviewed or not) (21 CFR §170.30(b)) and there can be no genuine dispute regarding the chemical's safety [3]. If an additive was in common use before 1958, safety may be based on that experience rather than scientific procedures (21 USC §321(s)).

In 1969, President Richard Nixon directed FDA to use updated scientific procedures it had developed to reassess the safety of food additives and GRAS substances that the agency had previously approved [3,4]. In 1977, in the midst of this review, FDA told the U.S. Senate that it "intended to extend the concepts of retrospective

**Abbreviations:** FDA, U.S. Food and Drug Administration; EPA, U.S. Environmental Protection Agency; TOR, Threshold of Regulation; FCS, Food Contact Substances; GRAS, Generally Recognized as Safe; FEMA, Flavor and Extract Manufacturers Association; EAFUS, Everything Added to Food in the United States; CASRN, Chemical Abstract Service Registry Number; PAFA, Priority-based Assessment of Food Additives.

<sup>☆</sup> This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

\* Corresponding author. Tel.: +1 317 442 3973; fax: +1 202 552 2299.

E-mail addresses: [tneltner@gmail.com](mailto:tneltner@gmail.com) (T.G. Neltner), [halger@pewtrusts.org](mailto:halger@pewtrusts.org) (H.M. Alger), [ldnyjEL@gmail.com](mailto:ldnyjEL@gmail.com) (J.E. Leonard), [drmvma@gmail.com](mailto:drmvma@gmail.com) (M.V. Maffini).

safety assessment of GRAS substances to potentially all chemicals that are added (directly or indirectly) to food in the United States” [5]. The agency cited changes in population exposure and advances in toxicological testing since the early 1960s when many of the additives were first approved.

As a result of this effort, in the late 1970s and early 1980s, FDA was at the leading edge of toxicological testing for chemical additives. The main outcomes were the publication in 1982 of its landmark “Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food”, also known as the “Redbook” [6], and the development of the Priority-based Assessment of Food Additives (PAFA) system [5] that included a database with toxicological information on direct additives (those added directly to food).

As part of this process, FDA established three “Concern Levels” (1, 2, and 3 with 3 being the most significant concern) for direct additives and color additives and developed recommended minimum toxicity tests for each level. The Concern Levels are based on a combination of estimated exposure and a prediction of the toxicity based on structural similarity to chemical with known hazards. The agency uses the levels to determine “the extent and type of basic toxicological testing of an additive” [6,7]. For instance, genetic toxicity tests and short-term toxicity tests with rodents are recommended for Concern Level 1 chemicals. FDA recommends additional studies including reproduction and developmental toxicity for Concern Levels 2 and 3, and human studies for Level 3. These recommendations remain in FDA’s guidance today [7].

The Redbook [6] recommended how the toxicology studies tests should be conducted. These tools were designed to help the agency and stakeholders establish clear expectations for what toxicology data the agency would need to make safety decisions.

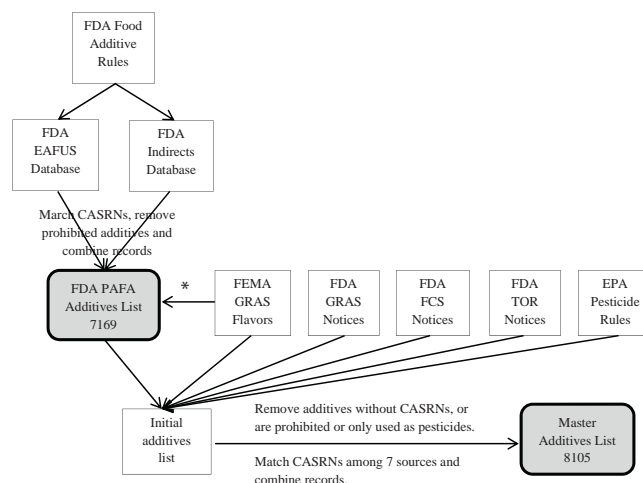
Pursuant to its commitment to the U.S. Senate, FDA also undertook a sustained effort to develop a strategy to reassess food additives and GRAS substances and documented its analysis in a series of peer-reviewed journal articles from 1981 to 1986 [8–10]. It did not evaluate indirect additives (chemicals used in packaging or as food contact substances (FCS)) “partly because they pose special problems of identification and exposure assessment” [8].

In the process of developing PAFA and the strategy, FDA scientists evaluated the available toxicological information for 1586 direct additives [8]. Their search included information FDA already possessed (whether published or unpublished) and data from the National Institute of Occupational Safety and Health’s (NIOSH) *Registry of Toxic Effects in Chemical Substances* (RTECS) database. Agency scientists only considered studies with ‘toxicological feeding information’ (i.e. studies where an animal or a human ingested a chemical regardless of duration or purpose), finding this information on 658 of the chemical additives [8]. In addition, FDA scientists concluded that only 159 (10% of the total) underwent more than a single dose toxicity test and had sufficient information to estimate the lowest effect level in any species tested [9]. Since the 1980s, FDA has continued to maintain the PAFA database but only include those additives it specifically approves through the formal rule-making process. Most FDA chemical safety reviews since 2000 do not go through this process.

Given FDA’s finding and the ongoing expansion of the additives market, the present study was undertaken to analyze how many chemical additives currently allowed in human food have published feeding studies.

## 2. Materials and methods

The authors conducted this research using a combination of Microsoft Access 2010 and Excel 2010 to compile information. To identify chemical additives, we used Chemical Abstract Service



**Fig. 1.** Resources used to identify allowed chemical additives. Shaded boxes indicate sources of chemical additive information used in the analysis.

\*Includes some chemical additives on FEMA GRAS flavors list.

Registry Numbers (CASRN); these are assigned to unique chemicals by the American Chemical Society. If the resource we used to develop the list provided another identification number instead of a CASRN, we used that number. Below is a brief description of the methods.

### 2.1. Step 1: Develop master additives list

We imported the following lists or databases (see Fig. 1) of chemicals allowed to be added to food and merged them into a table.

- FDA’s Priority-based Assessment of Food Additives (PAFA) database provided by FDA on Oct. 15, 2012 containing chemical information on:
  - Direct additives in its “Everything Added to Food in the United States” (referred to as EAFUS additives) database; and
  - Indirect additives in its “List of Indirect Additives Used in Food Contact Substances” (Indirects) database.

We removed chemicals that FDA had prohibited from use in food. There were 26 in “Everything Added to Food in the United States” (EAFUS) list [11] and 5 in the “List of Indirect Additives Used in Food Contact Substances” [12] (Arvidson, Personal Communication)].

- Notifications received and posted on the FDA’s website as of Feb. 7, 2012. For each program, we reviewed the information available on FDA’s website and captured the chemicals covered by the notice and, where available, CASRNs for those chemicals. We reviewed:
  - GRAS Notifications (GRN) [13]: Notices from food manufacturers and FDA response letters posted at FDA’s online database for 410 GRNs received from 1997–2011.
  - FDA’s Food Contact Substance Notifications (FCN) [14]: Summaries posted on online database for the 773 notices received since 2000 to which the agency had no objection.
  - FDA’s Threshold of Regulation Submissions (TOR) [15]: Summaries posted online for 105 exemption requests granted by FDA since 1996.

- Environmental Protection Agency's (EPA) pesticides with tolerances or exemptions in 581 sections at 40 CFR Part 180 [16] as of Jan. 1, 2012.
- Flavor and Extract Manufacturers Association's (FEMA) [17] list of GRAS substances provided by the organization as reported in its 25 GRAS publications since 1963 (Gavin, Personal Communication) on Dec. 15, 2011.

We generated the master additives list by refining the table as follows:

- Used National Institute of Standards and Technology (NIST) search engine [18] to identify CASRN for simple chemical names for chemical additives without CASRN or other identification number (provided by FDA or FEMA);
- Removed chemicals additives that still lacked CASRN or other identification number but kept those that FDA and FEMA assigned because they were in FDA's database and they were used in other documents;
- Combined information from the same number; and
- Removed chemicals used only as pesticides and kept those also approved to be used as food additives.

## 2.2. Step 2: Identify sources of toxicological information

We used three sources of toxicology information:

- FDA's PAFA database containing toxicology information for EAFUS additives as of Oct. 15, 2012. It contains published and unpublished studies and includes results from:
  - Acute lethal dose 50 (LD50) studies: study designed to calculate the amount of chemical needed to kill 50% of the study population, Oral studies: study designed to estimate the no-observed-adverse-effect-level (NOAEL), relevant to estimate the safe level of exposure; and
  - *In vitro* tests for genetic toxicity: designed to test for mutagenicity.
  - Minimum toxicity testing levels assigned by FDA based on levels of concern.

We also confirmed that there is a one-to-one correspondence between "Doc#" (FDA's key for each chemical) and CASRN; matched CASRN among the six "functions" identified by FDA (GRAS, flavor, indirect, direct, color or prior-sanctioned); and combined information of records with the same CASRN.

In addition to the files containing toxicology information, we identified:

- FDA's grades the completeness of many of the oral studies. The lowest grade is a "C" which indicates it does not meet the agency's core standards.
- "A" grade means it meets its "Toxicological Principles for the Safety Assessment of Food Additives", also known as the Redbook, standards.
- "B" grade when they met some, but not all of FDA's basic standards.
- "C" grade means it does not meet basic standards.
- Seven formats referring to sources that appear to be inconsistent with those used to identify articles in published journals. These formats start with the letters: ASP, CAP, FAP, FEM, FMF, GRM, or GRP. We considered these to be unpublished studies. They were most likely submitted to FDA as part of a notice or petition.

- Accelrys' "Toxicity" database (Accelrys) as of Feb. 29, 2012. This database contains published toxicology information from more than 114,000 studies in 3770 publications on more than 174,000 chemicals from three sources (Host, Personal Communication):

- Registry of Toxic Effects of Chemical Substances (RTECS) built and maintained by National Institute of Occupational Safety and Health (NIOSH) from 1971 to 2000. Since 2001, the private firm, Accelrys, has updated the information using the same data selection criteria and rules used by NIOSH [19].
- U.S. National Cancer Institute's Chemical Carcinogenesis Research Information System (CCRIS) with over 9000 chemical records with carcinogenicity, mutagenicity, tumor promotion, and tumor inhibition test results [20].
- GENE-TOX by EPA with test data, resulting from expert peer review of the open scientific literature on over 3000 chemicals [20].

- U.S. National Library of Medicine's (NLM) TOXLINE database of more than 4 million published bibliographic references that include PubMed and MEDLINE as of March 13, 2012 [21].

## 2.3. Step 3: Compare master additives list to PAFA and Accelrys toxicology information

We defined feeding studies to mean both oral (as defined by FDA and described in Section 2.2 Step 2) and LD50 where the exposure was via ingestion (including gavage). Although there is a broad spectrum of feeding studies with different duration and endpoints, we focused on the route of administration only because these were the two categories used by FDA in its database.

As mentioned above, PAFA only has toxicology data for EAFUS additives. We did the following comparisons to the Master Additives List:

- Matched chemical additives with Accelrys' toxicology data. We provided Accelrys with the names and associated CASRN or identification numbers to match against its "Toxicity" database. The firm provided us with a list of chemicals for which there is a record of toxic effect by feeding only or any route of exposure.
- Matched EAFUS chemical additives with PAFA toxicology data, identifying chemicals that had data in either oral or LD50 studies.
- Identified those direct additives in EAFUS that FDA has assigned a Concern Level 2 or 3 in PAFA and matched them with PAFA reproductive and developmental toxicology data, identifying chemicals that had data in either study. In its guidelines, the agency recommends that chemicals that fall under any of these levels are tested for reproductive and developmental toxicity [7]. None of this information was available in the other databases.

## 2.4. Step 4: Compare Accelrys results to TOXLINE and PAFA toxicology information

We recognize the possibility that Accelrys may have missed published feeding studies. Therefore, we used the U.S. National Library of Medicine's TOXLINE search engine [21] to identify whether feeding toxicology studies could be identified where Accelrys did not. We used the following process to conduct the review:

- Divided our Master Additives List into two parts: EAFUS additives (to compare to PAFA) and non-EAFUS additives.
- Refined each list to include only those chemicals that did not have information from a feeding study in Accelrys. We limited

the search to identify the rate of false negatives for feeding data in the Accelrys review.

3. Selected a sample size sufficient to be 95% confident that less than 10% of the chemicals without feeding studies results in Accelrys had feeding studies in TOXLINE (a desired confidence interval of 10%) for each list. We used the Macorr Research Solutions calculator [22].
4. Developed a random selection of chemical additives using Excel.
5. Entered the CASRN and selected “oral” as the route of exposure for each additive in TOXLINE Advanced search engine [21].
6. Searched using the additive’s name where a CASRN search returned an error in TOXLINE (where FDA, FEMA, or another entity assigned numbers to some additives). If name search yielded no results, then we looked for a true CASRN for the chemical using National Institute of Standards and Technology search engine and repeated the TOXLINE search using that CASRN.
7. Recalculated confidence interval based on the results from the searches.
8. Used the following equation to calculate the results:

Estimated number of additives lacking publicly-available feeding studies in TOXLINE = number of additives without feeding study results in Accelrys  $\times$  (1 – confidence interval – percent of random sample found to have feeding studies in TOXLINE).

#### 2.5. Estimating the frequency of references to FDA’s minimum recommended testing

We reviewed the 410 GRAS notices available from FDA’s GRAS Notice Inventory as of Feb. 7, 2012. For each of them, we searched for the following key terms:

1. Reproductive toxicity
2. Developmental toxicity
3. Concern levels or Levels of Concern
4. Redbook or Toxicological Principles (a short version of the official title Toxicological Principles for the Safety Assessment of Food Ingredients)

We listed all GRAS notices with their corresponding link to FDA’s inventory website and recorded the frequency of appearance of each of these terms in each notice. Then, we calculated the percentage of notices that made a reference to any of the following:

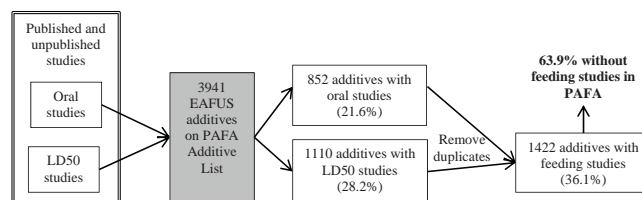
1. Performing Reproductive Toxicity testing;
2. Performing Developmental Toxicity testing;
3. Identifying the Concern Level of the chemical; and
4. Following the Redbook toxicology studies recommendations.

### 3. Results

#### 3.1. Analysis of lists of additives

FDA’s PAFA database contained chemical information on 7169 chemical additives from its on-line EAFUS and Indirects databases (see Supplementary materials database). Most of the chemicals were identified by CASRNs. However, FDA assigned a number for 1367 (19%) of these chemicals that follows the style of CASRNs [8].

The master additive list contained 8105 chemical additives with CASRNs or other identification numbers drawn from seven resources (see Supplementary materials database). Fig. 1 depicts the analysis and provides the total number of chemicals for PAFA and the Master Additives List. The list does not include 501 additives without codes provided by the resource or with readily identifiable CASRNs (80% from FDA notification programs and the balance



**Fig. 2.** Number of EAFUS additives with feeding toxicology studies in PAFA. Abbreviations: EAFUS: Everything Added to Food in the United States; PAFA: Priority-based Assessment of Food Additives; LD50: Lethal Dose to kill half of study population.

from EPA pesticide rules) and 384 chemicals used exclusively as pesticides.

The master additive list contained 936 chemical additives not on EAFUS or Indirect lists in PAFA: 344 on FEMA only; 128 on GRN only; 399 on FCN only; 53 on TOR only; 4 on both FEMA and GRN; and 8 on both FCN and TOR. Each of the three FDA notification programs identified one pesticide not listed in PAFA.

#### 3.2. Analysis of PAFA toxicology information for EAFUS additives

PAFA contains toxicology information for the 3941 direct additives listed in the EAFUS (referred to as EAFUS Additives) (see Supplementary materials database). These are chemicals allowed to be added directly to food at some stage of food production or processing as opposed to those only allowed to come in contact with food (known as indirect additives or food contact substances (FCS)).

The toxicology information is separated into two types of feeding studies: oral and LD50. We found 852 (21.6%) of EAFUS additives had oral studies, 1110 (28.2%) had LD50 studies, and 1422 (36.1%) have one or both. In other words, 63.9% had no feeding data (Fig. 2). Table 1 summarizes the number of EAFUS additives with oral or LD50 studies based on the additive’s function assigned by FDA in PAFA.

For the 3941 EAFUS additives, only 263 (6.7%) had reproductive toxicology and teratology data, and two had developmental toxicology measurements. In PAFA, FDA assigned 1136 EAFUS additives as Concern Levels 2 or 3. For these levels, FDA recommends reproduction studies and developmental toxicity studies among others [7]. Of these 1136 additives, 137 (12%) had reproductive or developmental toxicity data.

For the 1422 chemicals with feeding data, we found 5093 studies:

- 1286 LD50 studies 39% of which were unpublished (submitted to FDA without being made publicly available);
- 3807 oral studies 32% of which were unpublished;
- Of the 3807 oral studies, FDA graded studies based on whether they conform to its toxicological guidance to industry:
  - 7% conformed to guidance;
  - 16% met only core standards (standards used by agency before the 1982 guidance [5]);
  - 47% do not meet the core standards; and
  - 30% were ungraded.

Of the 852 EAFUS additives with an oral study, 293 (34%) had only studies that FDA graded as not meeting its core standards or guidance. FDA also included overall comments on the toxicological data such as:

- “No tox data” for 784 (19.9%) chemicals; and
- “Data Insufficient” for 272 (6.9%) chemicals.



**Table 1**

Number of Everything Added to Food in the United States (EAFUS) additives with feeding studies in priority-based Assessment of Food Additives (PAFA) percentage compares number to total additives with same function.

| Function <sup>a</sup>              | No. with an oral study | No. with a LD50 study | Total EAFUS additives |
|------------------------------------|------------------------|-----------------------|-----------------------|
| Direct                             | 677 (20.9%)            | 959 (29.6%)           | 3238                  |
| Flavor                             | 511 (17.3%)            | 866 (29.4%)           | 2950 <sup>**</sup>    |
| GRAS                               | 566 (19.3%)            | 857 (29.2%)           | 2938                  |
| Color                              | 65 (59.6%)             | 51 (46.8%)            | 109                   |
| Prior-sanctioned <sup>***</sup>    | 12 (14.6%)             | 13 (15.9%)            | 82                    |
| At least one of the five functions | 852 (21.6%)            | 1110 (28.2%)          | 3941                  |

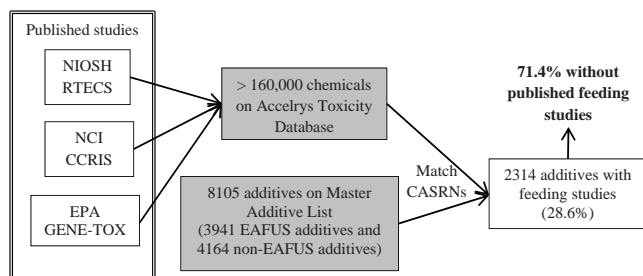
GRAS = Generally recognized as safe.

LD50 = Lethal dose to kill half the study population.

<sup>a</sup> Chemicals typically have multiple functions.

<sup>\*\*</sup> FDA designates more chemicals as flavors than are on the Flavor and Extract Manufacturers Association's GRAS Flavor list.

<sup>\*\*\*</sup> Prior-sanctioned refers to chemical additives approved by the federal government prior to 1958.



**Fig. 3.** Number of chemicals in master additive list with feeding toxicology data in Accelrys.

Abbreviations: NIOSH: National Institute for Occupational Safety and Health; RTECS: Registry of Toxic Effects of Chemical Substances; NCI: National Cancer Institute; CCRIS: Chemical Carcinogenesis Research Information System; EPA: U.S. Environmental Protection Agency; GENE-TOX: Genetic toxicity; EAFUS: Everything Added to Food in the United States; CASRN: Chemical Abstract Service Registry Number.

### 3.3. Analysis of Accelrys toxicology information

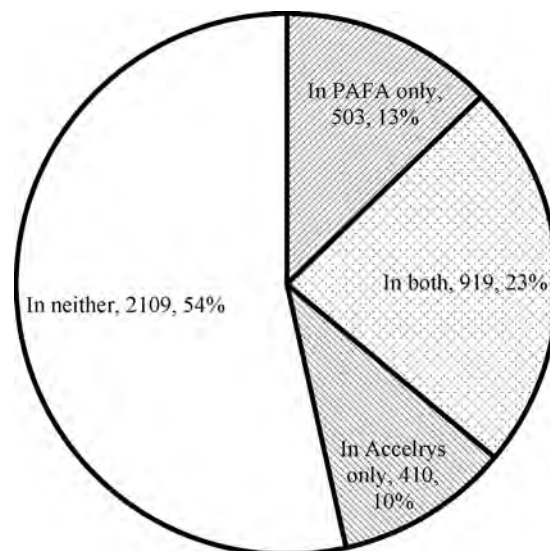
We found that 28.6% of the 8105 chemical additives on the master additive list had feeding study data in the Accelrys "Toxicity" database of published studies (Fig. 3). We did not ask Accelrys to distinguish between oral studies and LD50 studies. (see Supplementary materials database)

Table 2 provides the results for each of the resources used to identify chemical additives. For EAFUS additives, 66.3% lacked toxicology data, which is similar to the 63.9% lacking studies in PAFA. For non-EAFUS additives, 76.3% lacked feeding study data. We included the number of additives with any study results, not just feeding studies, to provide context. Note that FDA does not recommend feeding studies for food contact substances when estimated exposure is low [23]. It is important to note that Accelrys did not find any feeding studies for the 1380 chemical additives assigned a number by FDA or FEMA.

### 3.4. Comparison of Accelrys to TOXLINE

Recognizing that Accelrys may be missing studies, especially where FDA or FEMA assigned an identification number, we checked whether the same chemical additive without feeding data in Accelrys also lacked information in NLM's TOXLINE. Our evaluation provided the following results:

- **EAFUS Additives:** Accelrys did not identify feeding studies for 2612 additives or 66.3% of all EAFUS additives. We randomly selected 96 chemical additives from these and searched TOXLINE for feeding studies. Of the 96, 31 additives had numbers assigned by FDA. Therefore, we searched using variations of the chemical names. We found feeding studies for 11 (11.5%) of the 96. The recalculated 95% confidence interval was 6.4%. Using the



**Fig. 4.** Percentage of EAFUS additives with and without feeding toxicology studies in PAFA and Accelrys databases.

Abbreviations: EAFUS: Everything Added to Food in the United States; PAFA: Priority-based Assessment of Food Additives.

equation described in Section 2 Step 4, we estimated that at least 54% of EAFUS additives do not have published feeding studies in TOXLINE.

- **Non-EAFUS Additives:** Accelrys did not have feeding studies for 3179 additives or 76.3% of all non-EAFUS additives. We randomly selected 117 additives from these and searched TOXLINE for feeding studies. Of the 117, 29 additives had numbers assigned by FDA. Therefore, we searched using variations of the chemical names. We found feeding studies for only 9 (7.7%) of the 117. The recalculated 95% confidence interval was 4.8%. Using the equation described in Materials and Methods Step 4, we estimated that at least 67% of non-EAFUS additives do not have published feeding studies in TOXLINE.

Overall, we can be 95% confident that at least 62% of the chemicals on the Master Additive List do not have published feeding studies in TOXLINE.

### 3.5. Combined Accelrys and PAFA toxicology information for EAFUS additives

We found that 54% of EAFUS additives lack feeding studies in both Accelrys and PAFA databases (Fig. 4).

PAFA and Accelrys both draw from all published studies. However, PAFA also includes unpublished studies. In addition, PAFA uses identification numbers instead of CASRNs for 17% of EAFUS

**Table 2**  
Number of chemical additives on Master Additive List with studies in Accelrys database (percentage compares number to total additives from same resource).

| Resource used to identify additive <sup>a</sup>                  | No. with any study results | No. with any feeding studies | Total additives from resource |
|--|----------------------------|------------------------------|-------------------------------|
| PAFA database  |                            |                              |                               |
| Everything Added to Food in United States (EAFUS) Additives      | 2568 (65.2%)               | 1329 (33.7%)                 | 3941                          |
| Indirects Additives  | 1414 (43.7%)               | 810 (25.1%)                  | 3233                          |
| FDA notification programs  |                            |                              |                               |
| Generally Recognized as Safe (GRAS) Substances                   | 131 (68.6%)                | 73 (38.2%)                   | 191                           |
| Food Contact Substance (FCS)                                     | 352 (54.5%)                | 202 (31.3%)                  | 646                           |
| Threshold of Regulation (TOR) Substances                         | 76 (76.8%)                 | 46 (46.5%)                   | 99                            |
| Flavor and Extract Manufacturers Association (FEMA) GRAS Flavors | 2059 (75.3%)               | 1023 (37.4%)                 | 2735                          |
| At least one of the resources                                    | 4364 (53.8%)               | 2314 (28.6%)                 | 8105                          |

FDA = U.S. Food and Drug Administration.

<sup>a</sup> Chemicals may appear on more than one resource.

additives. Of the 503 EAFUS additives with feeding studies only in PAFA:

- 178 (35%) had only unpublished studies;
- 115 (23%) had identification numbers assigned by FDA; and
- 282 (56%) had toxicology studies other than feeding in Accelrys.

### 3.6. Consistency of results across all three toxicology information sources

Our analysis indicates that fewer than 38% of the chemical additives (direct and indirect) regulated by FDA have been the subject of a published feeding toxicology study. Table 3 provides the results for each of the three sources of toxicology information used in our analysis.

### 3.7. Frequency of references to FDA's minimum recommended testing

The 327 GRAS notices provide a window into whether additive manufacturers and FDA consistently follow the agency's guidelines regarding the minimum toxicity tests to be performed for safety evaluation of direct additives.

We found that:

1. (1%) notices assigned the chemical additive a Concern Level (a critical step to determining necessary testing according to FDA recommendations);
2. 75 (23%) notices reported results for developmental toxicity studies;
3. 120 (37%) notices reported results for reproductive toxicity studies; and
4. (<1%) notices assigned a Concern Level and reported results for both reproductive and developmental toxicity studies.

## 4. Discussion

Almost two-thirds of chemical additives appear to have been declared safe for use in food without the benefit of being fed to an animal in a controlled toxicology study. For the chemicals added directly to food that are listed in EAFUS, the results are better but still less than half have any feeding studies. Interestingly, there was excellent correlation among the three distinct toxicology information sources used in the analysis (Table 3). Where the agency recommended reproductive or developmental toxicity testing only one in eight had any data.

Virtually all EAFUS additives were initially allowed in food by FDA or FEMA before 1997. Since then, additive manufacturers have voluntarily notified FDA of their GRAS safety decisions of 191 new

direct chemical additives with CASRNs through the agency's GRAS notification program. These do not appear in EAFUS [1]. We found that more than 60% of these chemicals were approved by the additive manufacturer and reviewed by FDA without a published feeding study (Table 2), despite a requirement at 21 CFR 170.30 that the GRAS determination be based on a published study (regardless of route of administration).

In order to avoid overestimating the data gap, our estimates are based on all feeding studies including LD50 studies, which have limited relevance to chemical additive safety. Knowing how much it takes to kill half a study population may be useful to design additional studies but not to identify how much consumers can eat without harm. Oral studies (those designed to estimate a NOAEL) are the most relevant to determine a safe level of exposure. Yet, we found that 78.4% of EAFUS additives lacked oral studies in FDA's toxicology database (Table 1).

These results are consistent with FDA's own analysis of almost 1600 EAFUS additives published in 1985. The agency found that 90% had insufficient toxicological data [9]. We believe that the difference between our findings and FDA is that the agency's analysis excluded studies it graded as inadequate. In contrast, we included all studies without assessing their adequacy, since such a review is best done by the agency.

### 4.1. What are the limitations to the analysis?

Although our findings strongly suggest a substantial data gap, we acknowledge that our methodology has limitations, including:

- The three toxicology resources may not capture the entire universe of studies, particularly unpublished ones. PAFA contains about 5000 feeding studies. Accelrys has about 114,000 studies of all types (Host, Personal Communication) and TOXLINE provides access to more than 4 million [20]. We were not able to identify other sources that were as comprehensive.
- Review studies such as the Scientific Literature Reviews published by the National Technology Information Service were not consistently included by NIOSH in RTECS and, therefore, may not all be in Accelrys. Since the flavor industry relied heavily on these reviews, the percentage reported for these chemical additives is likely understated.
- Accelrys and TOXLINE only contain published studies drawing heavily from scientific journals. We offset this limitation with unpublished studies in PAFA for EAFUS additives that FDA received through food additive petitions or other means. We did not seek unpublished studies from FDA that were not described in PAFA or from other organizations.
- We did not conduct a chemical-by-chemical search in TOXLINE. Instead, we calculated a representative sample size of chemical additives without a feeding study in Accelrys. If the CASRN and

**Table 3**

Percentage of chemical additives with feeding toxicology studies.

| Source of toxicology information                             | EAFUS additives <sup>*</sup> | Non-EAFUS additives <sup>**</sup> | Chemical additives on master additive list |
|--|------------------------------|-----------------------------------|--|
| FDA's Priority Assessment for Food Additives (PAFA) database | 36.1% (Figure 2)             | Not applicable                    | Not applicable                             |
| Accelrys' "Toxicity" database <sup>***</sup>                 | 33.7% (Table 2)              | 23.7% (Table 2)                   | 28.6% (Figure 3)                           |
| Accelrys and PAFA combined                                   | 46% (Figure 4)               | Not applicable                    | Not applicable                             |
| National Library of Medicine's TOXLINE database              | <46%                         | <33%                              | <38%                                       |

FDA = U.S. Food and Drug Administration.

EAFUS = Everything Added to Food in the United States.

<sup>\*</sup> 3941 chemicals in EAFUS allowed to be added directly to food.<sup>\*\*</sup> 4169 chemicals with Chemical Abstract Service Registry Numbers (CASRNs) not in FDA's EAFUS database that are:

- 1) On its indirect additives database;
- 2) Reviewed by FDA through one of its three notification programs; or
- 3) Designated as "Generally Recognized as Safe" (GRAS) by the Flavor and Extract Manufacturers Association (FEMA).

<sup>\*\*\*</sup> Includes Registry of Toxic Effects of Chemical Substances (RTECS).

oral route of exposure were not properly designated, we would not have found feeding studies in TOXLINE.

- We did not find studies in Accelrys for chemical additives in PAFA that had identification numbers assigned by FDA or FEMA. We offset this limitation by supplementing Accelrys with toxicological information in PAFA as well as conduct searches in TOXLINE to estimate the 95% confidence level.
- Our analysis excluded almost 400 chemical additives identified through FDA's notification programs that lacked CASRNs. These are not in PAFA. Our experience with Accelrys indicated that finding results based on chemical names searches is difficult. We opted not to include these chemicals because they would have made the data gap appear larger than it might actually be.
- FDA did not assign Concern Levels to all the chemicals in EAFUS. We focused only on those that were clearly identified as level 2 or 3 because the agency recommended that additives with these levels be tested for reproductive and developmental toxicity.

Because FDA does not have a system to track usage of chemicals, it is unknown whether all of the allowed chemical additives are currently being used to process foods. In addition, there are an estimated 1000 chemical additives for which FDA has no information as to their names, uses, and in which foods they currently are used [1].

However, even considering these limitations, the major data gaps raise significant questions.

#### 4.2. How did we end up with so many chemical additives allowed in food without feeding studies?

We identified five reasons for the data gaps.

- Congress allowed FDA and food manufacturers to make GRAS determinations based on experience with the chemical's common use in food prior to 1958 rather than with scientific procedures.
- FDA approved thousands of chemical additives before it defined safety or issued guidance establishing how a safety determination should be done. In one case in which FDA approved hundreds of chemicals, the agency used 'unwritten assumptions' and made safety decisions on 'wish lists' submitted by industry, according to industry lawyers [24].
- FDA [23] and FEMA [25] rely on thresholds of exposure for food contact substances and flavors below which no feeding studies were expected.
- Once FDA or an additive manufacturer determines a chemical is safe to add to food, industry has little incentive to conduct additional studies. In addition, FDA does not systematically review its previous decisions. So if studies were not available for the initial approval, it is unlikely they would be conducted later.

- Academic researchers are unlikely to conduct studies to fill the gaps without a red flag that suggests a chemical poses a problem, and without funding available for such work.

#### 4.3. How did we end up with so many chemical additives allowed in food without reproductive and developmental toxicity studies?

We identified a few reasons:

- These studies are not explicitly required
- These studies are costly and time consuming.
- Our analysis of the GRAS notices submitted to FDA in the last 15 years strongly suggest that neither the manufacturer nor the agency's reviewers consistently follow the minimum recommendations established by FDA for direct additives. These findings indicate that the voluntary notification programs may not have contributed to filling the reproductive and developmental toxicology data gaps. This is disconcerting considering that, for chemical additives in PAFA, the agency had knowledge of the lack of studies through the notifications.

#### 4.4. Does the lack of toxicology studies for so many chemical additives represent a public health problem?

The law prohibits food manufacturers from using a chemical in food until its use is affirmatively determined to be safe (21 U.S.C. §321 and §348) by FDA or, in some cases, the additive manufacturer. This means "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use" (21 CFR §170.3(i)). If a chemical is added to food without this affirmative determination, the food is adulterated (21 USC §342(a)(2)(C)).

When estimated consumer exposure to FCSs is below exposure thresholds set by FDA, the agency has determined that feeding studies are not needed because these amounts are toxicologically insignificant [23]. This premise has been disputed, especially when FDA established the thresholds based on incomplete toxicology data and out-of-date scientific methods [26,27].

Professional judgment by competent scientists is central to safety determination and is needed to review the literature, estimate cumulative exposure, select the appropriate safety factor, and evaluate the available toxicological information. When applied in a consistent and transparent manner, it can help to fill in the gaps where a chemical is missing toxicology information. Usually, this is done by looking at the safety of similar chemicals, especially those with a long history of use, and inferring that the health impacts would be the same; however, given the substantial lack of toxicology data for chemical additives, extrapolation of the limited information to so many chemicals is disconcerting and may be

insufficient to ensure safety. Therefore, it may represent a public health problem.

#### 4.5. Why has FDA not addressed the problem?

FDA certainly is aware there are significant data gaps. Thirty years ago, FDA scientists knew that 90% of 1600 direct additives in EAFUS lacked sufficient data [10]. To date, the agency acknowledges its lack of toxicological information for as many as 1000 EAFUS additives [11].

We posit that FDA has not filled the gaps for three reasons.

1. It has determined that professional judgment is sufficient especially when exposures are estimated to be low (21 CFR §170.39) [23].
2. It has limited authority to require companies to tell the agency how much of a chemical additive is used or to demand additional testing.
3. If it wanted to conduct the research itself, its resources are limited.

In the 1980s, FDA did have a plan to reassess chemical additives with data gaps. The 1982 Redbook [6] contained the agency's framework for reassessment recognizing that additional toxicity information may be needed because either the original data were insufficient to make a final determination or "[a]dditives once approved do not always remain static relative to the exposure and toxicological criteria used originally to evaluate their safety" [5].

Five years later, an FDA scientist who later directed the food additive program recommended three criteria to determine when a safety decision needs to be reviewed [28].

- Toxicological information is either inadequate or nonexistent or clearly indicates the potential of the compound to produce toxic response of higher concern at doses that are low enough to bring the present margin of safety into question. In our analysis, we were unable to identify any program designed to fill the data gaps. In addition, our evaluation of the three notification programs that FDA launched in the 1990s suggests that shrinking the data gap has not been a priority (see Table 2). It is important to note that vast majority of FDA's reviews since 2000 are conducted through these three programs [1].
- **Chemical structure indicates higher than usual presumptive concern.** Through its Chemical Evaluation and Risk Estimation System (CERES), FDA has made a significant effort to develop computational and predictive methods to identify chemicals of concern [29]. It is scheduled to be released in 2014 and, unless it undergoes third-party validation, its use as the basis of regulatory decision-making is limited.
- **More than 100,000 pounds of the chemical is used in food annually.** At the time of the analysis, FDA relied on information voluntarily submitted by industry to the National Academy of Sciences (NAS). This program ended in 1987 [30].

FDA lacks the authority to track annual usage of additives (unlike EPA, which has such authority for industrial chemicals and pesticides), leaving the agency unable to apply this criterion. For example, pesticide manufacturers and processors must report annually to EPA on their production. In addition, pursuant to the Toxic Substances Control Act (TSCA), EPA requires manufacturers and processors of most organic chemicals to report detailed information (including the amount processed) every five years under its Chemical Data Reporting rule [31]. Unfortunately, by statute the information collected by EPA on chemical substances expressly excludes the amount used in food (15 U.S.C. §2602(2)(B)(vi)). If chemical additives to food were not excluded, EPA would have been

able to provide FDA with production data on more than 3776 (47%) additives subject to EPA's Chemical Data Report rule.

We believe the criteria are still relevant a quarter century after they were proposed.

#### 4.6. What should FDA do to fill the data gaps?

While feeding toxicology studies serve a critical function in safety determinations, it would be impractical to fill the data gaps solely through a massive new *in vivo* testing program applicable to all additives, especially in light of animal welfare concerns. Unlike in the 1980s when FDA established PAFA, today two powerful tools are being developed that, together, can be used to set priorities: computational toxicology and high-throughput *in vitro* testing.

Computational toxicology involves the use of sophisticated computer-based chemical structure modeling supported by existing toxicology data to identify chemicals with potential hazards. FDA used these methods to develop its Endocrine Disruptor Knowledge Base [32] that identifies potential endocrine disruptors, including more than 200 additives. The agency is also developing CERES (as discussed above). EPA and the National Institutes of Environmental Health Sciences (NIEHS) have similar efforts underway [33].

To increase the "ability to evaluate the large numbers of chemicals that currently lack adequate toxicological evaluation" [34], FDA has partnered with the National Toxicology Program (NIEHS/NTP), the NIH Chemical Genomics Center (NHGRI/NCGC), and the EPA's Office of Research and Development (EPA/ORD) to develop and implement 'Tox21' [33,35]. This program uses high-throughput *in vitro* testing and robotic equipment to run large numbers of chemicals across a wide range of concentrations and cell types to rapidly screen them for potential toxicity. While still early, preliminary analysis indicates this approach is useful to set priorities for additional evaluation. The success of this program "is expected to result in test methods for toxicity testing that are more scientifically and economically efficient and models for risk assessment that are more biologically based" [34] while reducing or replacing the use of animals in regulatory testing.

We identified two useful examples FDA could consider to address the information gaps:

- European Food Safety Authority (EFSA) reviews: The European Commission established EFSA in 2002 to serve as an independent source of scientific advice and communication on risks associated with the food chain. It directed EFSA to reevaluate by 2020 the safety of all chemical additives permitted for use in the European Union before 2009 [36]. To accomplish this, EFSA convenes expert panels and asks industry to provide missing information, but it cannot order testing. EFSA's published framework states that additives will continue to be used until their review is complete.
- EPA's High Production Volume (HPV) Challenge program [37]: In response to a report by the Environmental Defense Fund (EDF) [38], in 1998, EPA confirmed that there were significant gaps in the available toxicology information for HPV chemicals (those produced over 1 million pounds annually). Later that year, EPA, EDF and the American Chemistry Council "challenged" industry to voluntarily fill the information gaps. As a result, while certainly many data gaps remain [39], by 2004 companies sponsored more than 2200 chemicals and submitted about 6500 published and more than 8100 unpublished studies [40]. They also began testing to fill many of the remaining gaps [37]. EPA committed to using its authorities under TSCA to require testing for



unsponsored chemicals and to making the data available through its HPV Information System.

Both of these examples, however, would be difficult for FDA to adopt. Unlike EFSA, it lacks the authority and resources to establish a comprehensive reassessment program. Unlike EPA, it lacks the authority to systematically require testing.

## 5. Conclusions

A chemical additive is safe only if there is reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. With almost two-thirds of chemical additives lacking feeding toxicology and 78.4% of additives directly added to food lacking data to estimate a safe level of exposure and 93% lacking reproductive or development toxicity testing, it is problematic to assert that we know with reasonable certainty that all chemical additives are safe. Although FDA is aware of the problem, it lacks the authority and resources to fill the information gaps. Furthermore, once a chemical is approved, manufacturers have no incentive to add additional toxicology information because FDA neither has a reassessment program in place nor has authority to require additional testing.

Many of the decisions were made decades ago often based on extrapolations from limited data. Therefore, a program is needed to effectively and efficiently fill the significant information gaps to ensure public health is protected. This program should set priorities using various tools including *in vitro* and *in silico* (such as computational toxicology) methods complemented with the selective use of *in vivo* studies. In addition, the agency needs to seek authority to collect the information it needs and require testing when needed. EFSA's reassessment program and EPA's HPV Challenge program are two examples to be considered.

## Acknowledgements

This work was supported by The Pew Charitable Trusts. The authors thank Accelrys, Jim Woodruff (Environmental Management Institute), Erin Bongard, Juli Putnam, Erik Olson, Sean Cranshaw, and Neesha Kulkarni for their assistance and insightful discussion. Thanks also to Dennis Keefe of FDA for providing us with the PAFA database and to FEMA for providing us its GRAS list.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.reprotox.2013.07.023>.

## References

- Neltner TG, Kulkarni NR, Alger HM, Maffini MV, Bongard ED, Fortin ND, Olson ED. Navigating the U.S. Food Additive Regulatory Program. *Comp Rev Food Sci Food Saf* 2011;10:342–68.
- Congress. 1958. 85th U.S. Congress second session. H.R. report no. 2284. Congressional record Washington, DC.
- Degnan FH. FDA's creative application of the law: not merely a collection of words. 2nd ed. Washington, DC: FDLI; 2006.
- Nixon R. 1969. Special message to the congress on consumer protection. Available: <http://www.presidency.ucsb.edu/ws/index.php?pid=2299> [accessed March 15 2013].
- Smith M, Rulis AM. FDA's GRAS review and priority-based assessment of food additives. *Food Technol* 1981;71–4.
- FDA. 1982. Toxicological principles for the safety assessment of direct food additives and color additives used in food. U.S. Food and Drug Administration. U.S. Department of Commerce. National Technical Information Service. Springfield, VA.
- FDA. 2006. Guidance for industry: summary table of recommended toxicological testing for additives used in food. Food and Drug Administration. Available: <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocuments>
- RegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm054658.htm [accessed April 2, 2013].
- Rulis AM, Hattan DG, Morgenroth 3rd VH. FDA's priority-based assessment of food additives. I. Preliminary results. *Regulatory toxicology and pharmacology. Regul Toxicol Pharmacol* 1984;4(1):37–56.
- Rulis AM, Hattan DG. FDA's priority-based assessment of food additives. II. General toxicity parameters. *Regulatory toxicology and pharmacology. Regul Toxicol Pharmacol* 1985;5(2):152–74.
- Hattan DG, Rulis AM. FDA's Priority-Based Assessment of Food Additives: III. Specific toxicity parameters. *Regul Toxicol Pharmacol* 1986;6(2):181–91.
- FDA. 2011. Everything Added to Food in the United States (EAFUS). Food and Drug Administration. Available: <http://www.accessdata.fda.gov/scripts/cfn/fcnnavigation.cfm?rpt=eafuslisting> [accessed April 3, 2013].
- FDA. 2011. List of indirect additives used in food contact substances. Food and Drug Administration. Available: <http://www.accessdata.fda.gov/scripts/cfn/fcnNavigation.cfm?rpt=ialisting> [accessed March 22, 2013].
- FDA. 2013. GRAS notice inventory. Food and Drug Administration. Available: <http://www.accessdata.fda.gov/scripts/cfn/fcnNavigation.cfm?rpt=graslisting> [accessed March 22, 2013].
- FDA. 2013. Inventory of Effective Food Contact Substance (FCS) notifications. Food and Drug Administration. Available: <http://www.accessdata.fda.gov/scripts/cfn/fcnnavigation.cfm?rpt=fcslisting> [accessed March 22, 2013].
- FDA. 2013. Threshold of Regulation Exemptions: October 2000; updated January 2013. Food and Drug Administration. Available: <http://www.fda.gov/Food/IngredientsPackagingLabeling/PackagingFCS/ThresholdRegulationExemptions/default.htm> [accessed March 22, 2013].
- EPA. 2012. Indexes to part 180 tolerance information for pesticide chemicals in food and feed commodities. Environmental Protection Agency. Available: <http://www.epa.gov/pesticides/regulating/part-180.html> [accessed March 22, 2013].
- FEMA. 2012. About FEMA GRAS™ Program. Flavor and Extract Manufacturers Association. Available: <http://www.femaflavor.org/gras> [accessed March 22, 2013].
- NIST. 2011. NIST Chemistry WebBook—NIST Standard Reference Database number 69. Available: <http://webbook.nist.gov/chemistry/> [accessed March 15, 2013].
- Accelrys. 2013. Accelrys toxicity database. Available: <http://accelrys.com/products/databases/bioactivity/toxicity.html> [accessed March 15, 2013].
- NLM. 2011. National Library of Medicine Fact Sheets. Available: <http://www.nlm.nih.gov/pubs/factsheets/factsheets.html> [accessed March 15, 2013].
- NLM. 2012. Toxicology Literature Online (TOXLINE). National Library of Medicine. Available: <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE> [accessed March 15, 2013].
- Macorr. 2013. Sample size calculator. Available: <http://www.macorr.com/sample-size-calculator.htm> [accessed March 22, 2013].
- FDA. 2002. Guidance for industry: preparation of food contact notifications for food contact substances: toxicology recommendations. U.S. Food and Drug Administration. Available: <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm081825.htm> [accessed April 3, 2013].
- Sylvain Baughan J. 2002. What's under the coat of section 175.300? Available from: <http://www.khlaw.com/showpublication.aspx?Show=2293> [accessed March 22, 2013].
- Hallagan JB, Hall RL. Under the conditions of intended use—new developments in the FEMA GRAS program and the safety assessment of flavor ingredients. *Food Chem Toxicol* 2009;47(2):267–78.
- Select Committee on GRAS Substances (SCOGS). 1982. Insights on food safety evaluation. U.S. Department of Commerce. National Technical Information Service. Life Sciences Research Office, Federation of American Societies for Experimental Biology. Springfield, VA.
- Maffini MV, Alger HM, Olson ED, Neltner T. Looking back to look forward: a review of FDA's food additives safety assessment and recommendations for modernizing its program. *Comp Rev Food Sci Food Saf* 2013;12:439–53.
- Rulis AM. Safety assurance margins for food additives currently in use. *Regul Toxicol Pharmacol* 1987;7(2):160–8.
- FDA. 2012. About FDA: Chemical Evaluation and Risk Estimation System (CERES). U.S. Food and Drug Administration. Available: <http://www.accessdata.fda.gov/FDAtask/track-proj?program=cfsan&id=CFSAN-OFAS-Chemical-Evaluation-and-Risk-Estimation-System> [accessed March 15, 2013].
- FDA. 2006. Guidance for industry: estimating dietary intake of substances in food. U.S. Food and Drug Administration. Available: <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm074725.htm> [accessed April 2, 2013].
- EPA. 2013. Chemical data reporting. Environmental Protection Agency. Available: <http://www.epa.gov/cdr/> [accessed March 15, 2013].
- FDA. 2010. Endocrine disruptor knowledge base. U.S. Food and Drug Administration, Office of Science and Research. Available: <http://www.fda.gov/scienceresearch/bioinformaticstools/endocrinedisruptorknowledgebase/default.htm> [accessed March 15, 2013].
- Collins FS, Gray GM, Bucher JR. Transforming environmental health protection. *Science* 2008;319:906–7.
- NTP, NCCG, EPA, and FDA. 2008. Memorandum of understanding on high throughput screening, toxicity pathway profiling, and biological

- interpretation of findings. Available: [http://www.epa.gov/ncct/download\\_files/tox21/MOU\\_EPA-NTP-NCGC-FDA-Without-Signature-Page.pdf](http://www.epa.gov/ncct/download_files/tox21/MOU_EPA-NTP-NCGC-FDA-Without-Signature-Page.pdf) [accessed July 29, 2013].
- [35] EPA. 2013. Computational toxicology research Tox21. Environmental Protection Agency. Available: <http://www.epa.gov/ncct/Tox21/> [accessed March 15, 2013].
- [36] EFSA. 2012. Food additives. European Food Safety Authority. Available: <http://www.efsa.europa.eu/en/topics/topic/additives.htm> [accessed March 15, 2013].
- [37] EPA. 2012. High production volume challenge. Environmental Protection Agency. Available from: <http://www.epa.gov/hpv/> [accessed March 15, 2013].
- [38] EDF. 1997. Toxic ignorance: the continuing absence of basic health testing for top-selling chemicals in the United States. Environmental Defense Fund. Available: [http://www.edf.org/sites/default/files/243\\_toxicignorance.0.pdf](http://www.edf.org/sites/default/files/243_toxicignorance.0.pdf) [accessed March 15, 2013].
- [39] Denison RA. 2007. High holes, low marks. A final report card on the high production volume chemical challenge. Environmental Defense Fund. Available from [http://www.edf.org/sites/default/files/6653\\_HighHopesLowMarks.pdf](http://www.edf.org/sites/default/files/6653_HighHopesLowMarks.pdf) [accessed March 15, 2013].
- [40] EPA. 2010. Status and Future Directions of the High Production Volume (HPV) Challenge Program. Environmental Protection Agency. Available: <http://www.epa.gov/hpv/pubs/general/hpvestatr.htm> [accessed March 15, 2013].