

On Categorizations in Analyses of Alcohol Teratogenesis

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In biomedical scientific investigations, expositions of findings are conceptually simplest when they comprise comparisons of discrete groups of individuals or involve discrete features or characteristics of individuals. But the descriptive benefits of categorization become outweighed by their limitations in studies involving dose–response relationships, as in many teratogenic and environmental exposure studies. This article addresses a pair of categorization issues concerning the effects of prenatal alcohol exposure that have important public health consequences: the labeling of individuals as fetal alcohol syndrome (FAS) versus fetal alcohol effects (FAE) or alcohol-related neurodevelopmental disorder (ARND), and the categorization of prenatal exposure dose by thresholds. We present data showing that patients with FAS and others with FAE do not have meaningfully different behavioral performance, standardized scores of IQ, arithmetic and adaptive behavior, or secondary disabilities. Similarly overlapping distributions on measures of executive functioning offer a basis for identifying alcohol-affected individuals in a manner that does not simply reflect IQ deficits. At the other end of the teratological continuum, we turn to the reporting of threshold effects in dose–response relationships. Here we illustrate the importance of multivariate analyses using data from the Seattle, Washington, longitudinal prospective study on alcohol and pregnancy. Relationships between many neurobehavioral outcomes and measures of prenatal alcohol exposure are monotone without threshold down to the lowest nonzero levels of exposure, a finding consistent with reports from animal studies. In sum, alcohol effects on the developing human brain appear to be a continuum without threshold when dose and behavioral effects are quantified appropriately. *Key words:* alcohol, ARND, developmental disabilities, diagnosis, dose–response, dysmorphology, FAE, FAS, multivariate, risk, threshold. — *Environ Health Perspect* 108(suppl 3):421–428 (2000).

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In this article, we address a pair of categorization issues concerning the effects of prenatal alcohol exposure that have important public health consequences: the labeling of individuals as fetal alcohol syndrome (FAS) versus fetal alcohol effects (FAE) or alcohol-related neurodevelopmental disorder (ARND), and the categorization of prenatal exposure dose by thresholds. The practice of classification or categorization is common in biological and medical scientific investigations. Expositions of findings are conceptually simplest when they comprise comparisons of discrete groups of individuals or involve discrete features or characteristics of individuals. Consider the broad range of investigations into human developmental disturbances and disabilities of known or perhaps uncertain origin. The case of Down syndrome provides a useful baseline for consideration. The genetic cause of the syndrome is discrete: you can have either two or three copies of chromosome 21, not, for example, 2.92. And the consequences of the cause are also usefully considered discrete in the classification of individuals with the dysmorphic and behavioral characteristics of the syndrome. While there is variation in the extent or magnitude of the morphologic and behavioral characteristics evidenced in the syndrome, all those with the genetic defect manifest these characteristics to a substantial

extent, and the existence of the genetic defect can be reliably inferred from identification of these characteristics.

The benefits of categorization in terms of simple description become outweighed by limitations as one moves to studies involving dose–response relationships, as is the case in many teratogenic and environmental exposure studies. Issues arise in the measurement of the dose or cause of the developmental disturbance and also in the measurement of that disturbance. The benefits of categorization of response appear attractive: one can compare groups of people affected by exposure to certain levels of a teratogen to other groups of people not affected. And similarly, one may be able to discuss discrete levels of exposure that are necessary for effects to be manifest. There are obvious consequences of these categorizations for diagnostic practices, treatment, and public health policy. Furthermore, statistical analysis may be greatly simplified by these categorizations. Categorization may, for example, enable two-sample *t*-tests instead of possibly nonlinear dose-response regression analyses, or it may permit analysis in terms of contingency tables and risk of discrete events/effects. But categorization can be misleading, especially when a teratogenic cause must be measured in a multivariate manner to assess the timing and patterns of exposure

and when the morphological and behavioral effects are subtle.

Our subject here is alcohol teratogenesis, for which the behavioral effects are sometimes subtle but nonetheless have major public health consequences in terms of secondary disabilities such as mental health problems, disrupted school experience, and trouble with the law. We begin with an assessment of the public health consequences of a misplaced emphasis on the categorization or diagnosis of FAS vis-a-vis other characterizations of fetal alcohol-affected individuals. We then discuss the issues of categorization of exposure and the reporting of threshold effects in dose–response relationships and conclude with a comment on the public health implications of the possible decision procedures.

Categorizing FAS and Screening for Prenatal Effects of Alcohol

Background

An array of clinical manifestations was first described 25–30 years ago in young infants of three races born to chronically alcoholic mothers (1,2). Three broad categories of deficit emerged as the basis for a diagnosis of FAS: face, growth, and brain. Figure 1 depicts the characteristic face of FAS. Natural history studies reveal the increasing variability of the facial and growth characteristics with increasing age (4–10). These highlight the limited age range for using facial stigmata and even growth deficiency as criteria for the diagnosis

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of FAS. Since 1978, partial manifestations of these deficits have been described in offspring of alcoholic mothers. These have been termed fetal alcohol effects (FAE), a phrase sometimes preceded by "possible" or "probable" (11–13), or mild FAS (Type III) (14,15). More recently, the Institute of Medicine of the U.S. National Academy of Sciences has suggested a new and extended system of classifications for children affected by prenatal alcohol exposure (16). The classification alcohol-related neurodevelopmental disorder (ARND) essentially replaces the term FAE as used here to describe offspring with central nervous system (CNS) deficits but not all the physical features of FAS. Going from the level of the individual to the level of groups, epidemiologic studies examining the consequences of prenatal alcohol across the full range of exposures have revealed a broad spectrum of alcohol-related deficits in humans from birth through adolescence (17–24).

The teratogenicity of alcohol has been established by a vast experimental animal literature (25–28). This literature shows the importance for outcome of the dose, timing and conditions of exposure, as well as individual sensitivity of both mother and offspring, according to the tenets of teratogenic theory (29). However, pressing clinical concerns are emerging that must be resolved.

When the originally diagnosed FAS children were identified (1), their compromised intellectual development led others to the conclusion that mental retardation was ubiquitous in this birth defect, so that social support systems developed for the mentally retarded would suffice for their education and training. FAS was often described as the leading known cause of mental retardation, further implanting the syndrome within that domain. The preoccupation with the mental retardation aspect of FAS has contributed to disregard for the neuropsychological deficits of alcohol-affected children and adults with FAS/FAE who fail to meet conventional criteria for mental retardation (IQ < 70).

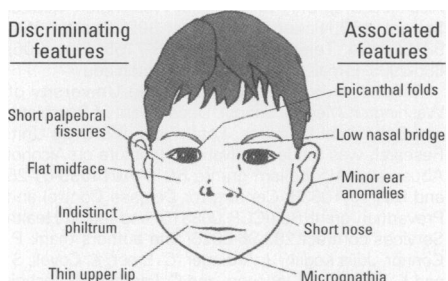


Figure 1. The face of fetal alcohol syndrome in the young child (23).

Behavioral and Neuropsychological Performance Comparisons of FAS and FAE Patients

Here we examine the question about the relevance and consequences of categorizing alcohol-related birth defects into FAS and other non-FAS alcohol-related effects. Is it still appropriate to draw a distinction between FAS and FAE? We present some data from a large fetal alcohol follow-up study conducted at the University of Washington in Seattle, Washington, over the past 25 years (30). A diagnosis of FAE was attributed to those who had a clear history of prenatal alcohol exposure and CNS dysfunction but did not manifest all of the physical features of FAS. All subjects had previously been examined and diagnosed by dysmorphologists experienced in FAS diagnosis and associated with the late David W. Smith of the University of Washington or one of his fellows. Examination of this large cohort of children and adults previously identified as having FAS or FAE reveals a surprising array of secondary disabilities, defined as those difficulties a child is not born with but that

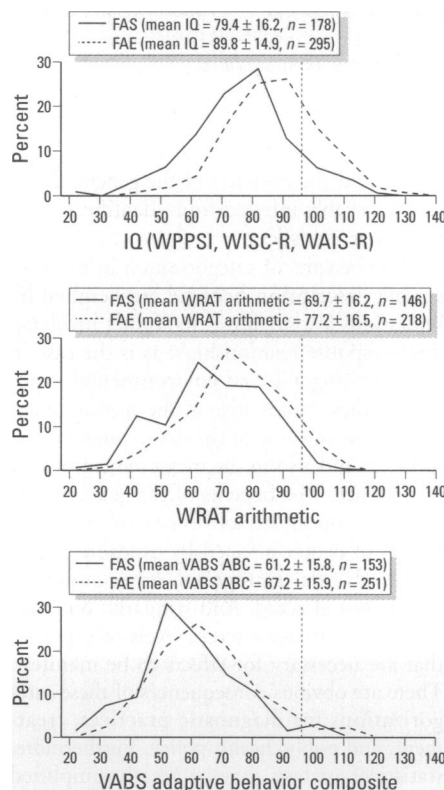


Figure 2. Distributions comparing FAS and FAE. (Top) IQ distributions. IQ scores from Wechsler IQ tests: Wechsler Preschool and Primary Test of Intelligence (31); Wechsler Intelligence Scale for Children—Revised (32); Wechsler Adult Intelligence Scale—Revised (33). (Middle) WRAT distribution. Wide Range Achievement Test (34) Arithmetic scores. (Bottom) VABS distribution. Vineland Adaptive Behavior Scale (35) Adaptive Behavior Composite Scores.

may result in part from the primary CNS dysfunctions inherent in the FAS or FAE diagnosis. Of 415 of these patients with FAS/FAE who were assessed via caretaker interview, over 90% had mental health problems and had sought professional help for these problems; 61% of the 253 who were adolescents and adults had been suspended or expelled, or had dropped out of school; and 60% had been in trouble with the law. Adolescents and adults categorized as FAE had higher rates on all these secondary disabilities than those categorized as FAS. Among the 90 adults studied, 83% were still living dependently and 79% were unable to maintain regular employment without major difficulties.

Do these two categorizations of alcohol-affected patients differ on the types of standard tests administered in our unit? Figure 2 characterizes the distributions of IQ, WRAT Arithmetic, and the Vineland Adaptive Behavior Score (VABS) composite scores for 178 patients with FAS and 295 with FAE.

The means of the FAS and FAE distributions are separated by 10 points (2/3 of a standard deviation) for IQ, 7 points for WRAT Arithmetic, and 6 points for VABS (the latter are less than one-half a standard deviation). Figure 3 (top graph) shows how the combined FAS and FAE distribution deviates from the normative distribution for IQ scores. If IQ < 70, the usual demarcation of mental retardation, were the sole criteria for service delivery,

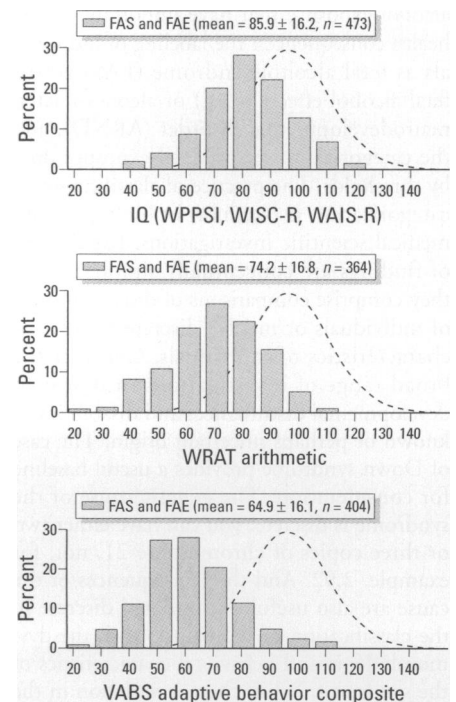


Figure 3. IQ, WRAT Arithmetic, and VABS Adaptive Behavior Composite: combined distribution for FAS and FAE compared to the normative.

only 27% of the patients with FAS and 9% of those with FAE would be eligible for services. Nonetheless, a minimal estimate of the birth prevalence of FAS in Seattle shows that FAS and FAE patients may account for about 1 in 20 cases of mental retardation by the IQ < 70 criterion. [Assume 2.5% of the population have IQ < 70, 2 standard deviations below the mean, and incidence rates of FAS and FAE for Seattle of approximately 2.8/1,000 and 6.3/1,000, respectively, as estimated from a large Seattle 1973–1974 birth cohort originating from a population-based consecutive sample of women in prenatal care by the 5th month of pregnancy (3). Then $0.27 \times (2.8/1000) + 0.09 \times (6.3/1000) = 0.0013$ or 0.13%; $0.13/2.5 \approx 0.05 = 1/20$.] Hagberg et al. (36,37) estimated by similar but entirely different methods that fetal alcohol effects might account for 8% of all cases of mental retardation (IQ < 70) in Sweden over the years 1966–1970 (though the overall rate of mental retardation in Sweden by their definition was only 0.7%). Although receiving developmental disabilities services from the state of Washington was a strong protective factor against secondary disabilities in this study (29), only a small number of the adolescents and adults actually received such services, mainly because they were not sufficiently retarded.

IQ is not the only behavioral dimension worthy of note in this population. Clinical descriptions of children, adolescents, and adults with FAS and FAE have revealed learning problems, particularly arithmetic and number processing problems (7,23,38), and a wide variety of behavioral problems (39–42). Figure 3 represents the combined FAS/FAE distributions for three of the most common types of psychological tests all scaled to a standard deviation of 15. Arithmetic scores of FAS/FAE patients are more deviant from the normative distributions than are IQ scores, and VABS are even more deviant: 14, 37, and 64% of our FAS/FAE subjects score lower than 70, 2 standard deviations below the mean on the normative distribution, for IQ, Arithmetic, and VABs, respectively.

A number of studies show that patients with FAS and with FAE experience neuropsychological problems (43–45). In particular, executive function (EF) deficits involving problems with judgment, reasoning, and organization (thought to be related to frontal lobe dysfunction) have been of interest in these patients because of their high level of adaptive behavior problems. We have recently conducted an EF study (46) on 30 adult males with FAS/FAE drawn from the large fetal alcohol follow-up study cited above (29). In general populations, variation of EF scores and many other neuropsychological assessments can often be explained largely by

differences in IQ. However, on a number of individual components of our EF battery, patients with FAS/FAE function, in general, scored worse than would be expected on the basis of their IQ scores alone. Figure 4 demonstrates this for the number of errors on the Wisconsin Card Sorting Test and number of correct responses on the Stroop Word Reading. Figure 4 also shows that the FAS and FAE subjects are perfectly intermingled and well discriminated from a sample of 392 subjects not having high prenatal alcohol exposure scores.

Covariates and Screening for Fetal Alcohol-Affected Individuals.

The problem with the FAS face, as an indicator of fetal alcohol-affected individuals, is poor sensitivity—there are too many false negatives (affected individuals without the FAS face characteristics who are thus often not identified). Research is needed to find substitutes for “the face” having high sensitivity while not losing specificity. The results demonstrated in Figure 4 are promising in this regard. Were there a clinical categorization of deficit for, e.g., the Stroop Word Reading Task, a substantial fraction of the patient group would be declared in deficit regardless of IQ. In Connor et al. (48), these and corresponding plots for all of the components of our EF battery underlie calculations of a simple model for two paths of effects of prenatal alcohol damage on EF, one direct and one indirect through the mediation of IQ. These suggest the calculation of an EF composite score that weights most heavily the individual components most indicative of direct effects. The component weights are simply the difference between the mean IQ-adjusted component score for the FAS/FAE subjects and the mean IQ-adjusted component score for the comparison or control group. The resulting weighted combination derived from IQ-adjusted scores is a composite that is far more specific for fetal alcohol effects than IQ or Adaptive Behavior. The major effects of environmental factors, including socioeconomic status and/or parental education, are expressed in full-scale IQ; by deemphasizing the path through IQ to identify an alcohol-specific composite outcome, it becomes largely unnecessary to carry out multiple regression adjustments of outcomes for environmental influences. For further discussion of the relationship of full-scale IQ with socioeconomic factors and a related adjustment for covariates via IQ filtering, see Streissguth et al. (22). For consideration of explicit measures of neuroanatomic damage in the identification of alcohol-specific neuropsychological effects, see discussion below and Bookstein et al. (49).

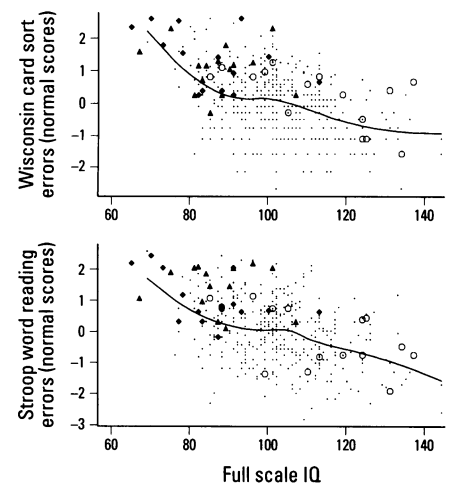


Figure 4. Full-scale IQ plotted against number of errors on the Wisconsin Card Sorting Task (top panel) and number of correct responses on the Stroop Word Reading task (bottom panel). Both scores have been re-expressed as “normal scores” (equivalent normal distribution percentiles) for analysis, with the Stroop score reversed in direction. Solid diamonds, solid triangles, and open circles, encode FAS, FAE, and control subjects, respectively. Dots represent a larger sample of control subjects from the Seattle Longitudinal Prospective Study (22) with low or no prenatal alcohol exposure. Suspected bias in sampling of control subjects for the FAS and FAE subjects explains the greater than expected deficits of these controls (open circles) in comparison with the prospective study controls (dots). The curves drawn represent nonlinear regressions computed from the control subjects using the “loess” function in the S-Plus statistical programming language (47).

Dose–Response Modeling and Thresholds for Prenatal Alcohol Exposure

The categorization of exposure in terms of thresholds for “safe” levels of maternal consumption, and more generally, methods of dose–response modeling, have, like the categorization of response, been problematic for the field of prenatal alcohol studies. The recent Institute of Medicine report on fetal alcohol syndrome stated that

Dose–response relationships are among the most critical issues in developmental toxicology, yet they are too often misunderstood, oversimplified, or simply neglected. (16)

In discussing a diagram relating hypothetical maternal and fetal dose–response effects the report notes that

. . . the relationship of maternal to fetal dose response effects is incompletely understood. Although the data strongly support a relationship of chronic high levels of maternal alcohol intake to the full FAS, what remains unclear is whether there is a continuum of dose–response effects ranging from anatomic and behavioral changes at low to moderate maternal

doses to full blown FAS at high maternal doses, or if there are two or more thresholds resulting in degrees of impairment in function and structural malformation. (16)

Victorin et al. (50) offer a useful summary of the methods of categorization in health risk assessment and their relevance for alcohol (not specifically prenatal alcohol exposure). On the subject of dose-response assessment, they note that toxic agents such as alcohol can have several toxic effects and that the critical effect or the critical organ should be identified. The critical organ in the context of prenatal alcohol exposure is, of course, the brain. See discussion below for further remarks on measurement of the brain in connection with the recent Institute of Medicine's definition of ARND (16).

In many contexts there is a level of exposure below which there is no enhancement of the chance of an adverse effect. Such a level is called the threshold. The underlying mechanism for a threshold at the level of an individual, applicable generally to nongenotoxic substances, is that multiple cells or cell components must be injured before an adverse effect is experienced and that injury must occur at a rate that exceeds the rate of repair. Victorin et al. (50) describe the conventional approach (supported by World Health Organization publications) to deriving safe exposure levels for food additives and environmental contaminants on the basis of studies providing "no observed adverse effect levels" (NOAELs) in animal and/or human studies. The first requirement is that the determination of an NOAEL must be based on the most sensitive indicator of toxicity. An empirically observed NOAEL is then divided by a factor to account for all the uncertainty inherent in the NOAEL, including consideration of sample size, variation in susceptibility in human populations, and in the case of animal studies, extrapolation to humans. Without knowing what the most sensitive human subgroups are, it is common to use uncertainty factors ranging from 10 for NOAELs from human studies to 100 for NOAELs from animal studies. This is discussed by Jacobson and Jacobson (51,52). In fact, in the case of the effects of prenatal alcohol exposure, we are unlikely to approach identification of the most sensitive indicator of damage without direct measurement of the most sensitive organ, the brain. This fact, together with the complexity of the variation of alcohol consumption over time in epidemiological studies, make the definition of dose so problematic that the application of the NOAEL methodology becomes essentially impossible. No argued alternative methodology has been proposed for the specification of safe levels.

In the 1996 symposium "New Directions in Fetal Alcohol Syndrome Research" (53), James West suggested that it is important to understand if alcohol acts in a stochastic manner, similar to mutagens or carcinogens, with decreasing but continuous risks, or instead in a discontinuous manner, similar to certain other environmental teratogens, with a threshold of damage. He argues that since alcohol apparently affects CNS development at all stages, it is highly unlikely that a single mechanism could be responsible for all of the varied effects that have been observed. Furthermore, if multiple mechanisms are involved, then it is almost certain that there is no single threshold for all fetal alcohol-induced damage.

A considerable amount of animal research suggests that thresholds will not be found at other than quite moderate, or in some case, the very lowest measurable levels of exposure, and the effects seen at these low levels are principally on the CNS. For example, Riley et al. (54) show a clear dose-response relationship for mean trials to criterion in a passive avoidance (response inhibition) test with rats in four dose groups defined by percent-ethanol-derived calories: 0, 8, 19, and 32%. The lowest nonzero dose group, 8%, had mean maternal blood alcohol concentrations of only 4 mg%. Vaglenova and Petkov (55) demonstrated the behavioral teratogenicity of ethanol in a rat model at a "very low dose of ethanol" (1 g/kg/day), which resulted in blood ethanol of 35 mg% throughout pregnancy from day 1. Zhou and colleagues (56) show that low-dose prenatal alcohol exposure in fetal mice (60–70 mg%) derails normal migration of 5-HT neurons, reduces their number by 20–30%, and disrupts the brain serotonin system, which has long-term implications for mental integrity. Goodlett and West (57) review many studies showing CNS effects induced by relatively low blood alcohol concentrations, some in the range of 50 mg% [under two drinks human equivalent (58); see also (16)]. Gestational alcohol exposure has particularly long-lasting effects on neurochemistry and neurophysiology. With exposure during the brain growth spurt (third trimester rodent models), many effects appear to be a linear function of the peak blood alcohol concentration attained. Further discussion and references for low-dose effects are provided in the Institute of Medicine report (16).

CNS damage, occurring relatively early, when the developing brain is the most vulnerable, is understandably debilitating due to the inability of neurons to multiply or replace themselves after the initial production period (59). Consuelo Guerri, in her discussion of the 1996 symposium cited above (53), noted that, despite the great plasticity

of the developing brain, it has limited repair capacity during development and therefore small disturbances in the correct formation of the different brain areas may result in behavioral deficits that may not be detected until school age or adulthood.

Proceeding to the literature on observational studies of humans, recent publications of Jacobson et al. (60) and Abel (61) would lead one to believe that issues of dose-response methodology have been oversimplified or neglected, as suggested also in the Institute of Medicine report. Jacobson et al. (60) state incorrectly that essentially all the prospective longitudinal studies of prenatal alcohol exposure have relied primarily on multiple regression or other correlational procedures that assume linear relationships between dose and effect and that will miss the effects of possible nonlinear or threshold effects in dose-response relationships. That is certainly not the case for most of the publications over the past 10 years from the Seattle longitudinal study (22,62), nor is it an accurate characterization of analyses reported by the Pittsburgh study (18). Abel (61) stands out in his disregard for the animal and human literature on dose-response effects cited above. He is appropriately critical of studies in the literature that have relied on measures of average alcohol intake per day for assessment of effects, but not all studies have made this error, and his proposal to change the name of fetal alcohol syndrome addresses no important problem.

We agree that it is inappropriate to address threshold effects, like any other dose-response relation, using the wrong measures of dose. Thus, it was surprising to find that Jacobson et al. (60) noted the importance of binge indicators of prenatal alcohol exposure and yet assessed a threshold effect in terms of average daily intake without apparently examining relationships with binge measures. Fortunately, neither Jacobson et al. (60) nor the authors of the Euromac studies (63) claim that their empirically determined thresholds on average daily consumption measures represent biologically meaningful indicators of safe levels of exposure.

Multivariate Analysis of the Dose-Response Relationship

In the context of alcohol teratogenicity, the identification of thresholds of exposure depends fundamentally on the choice of measures of dose and of outcome. Indeed, if the outcome considered is the "face of FAS," whether categorized or assessed quantitatively in terms of one or more morphometric measures, statistical analysis will certainly reveal thresholds, as FAS is manifest only at very high levels of alcohol exposure and only for a specific period of development. The face of

FAS is time delimited, as Sulik and Johnston (64) have shown, to exposure during a specific period of embryogenesis.

The complexity of patterns of human consumption of alcohol over the entire period of gestation greatly complicates any attempted discussion of low levels of alcohol exposure. The timing of exposure is important, with exposure having different consequences depending on the stages of neuroanatomic development. Is a history of one binge episode of, for example, five drinks to be considered low, in as much as there was only one, or high, in as much as the peak blood alcohol concentration was high? Animal and human literature have identified the importance of peak blood alcohol concentrations for both neuroanatomic and behavioral effects; see Clarren et al. (65) and the Institute of Medicine report (16) and references therein. An effective analysis of alcohol teratogenicity in humans therefore demands consideration of multiple measures of maternal alcohol consumption which, considered together, imperfectly indicate variation in timing and dose of actual prenatal alcohol exposure. But one must also recognize that a given score on, for example, a measure of average drinking behavior, such as average drinks per drinking occasion or average drinks per day, lumps together individuals with varying maximum number of drinks on any occasion, and vice versa. It is obviously not adequate to select a priori one convenient maternal alcohol consumption indicator and use only that one measure to predict outcomes or to assess possible thresholds. But this is a surprisingly common strategy.

At the same time, neurobehavioral response for dose–response analysis must be addressed from a similarly multivariate perspective. The critical effect, as referred to above, can never be known in advance; however one may try to define this effect, it is unlikely to be well characterized by any single neuropsychological test or by a conventional summary score from a standard neuropsychological test (such as a full-scale IQ score). Instead, only a multivariate strategy can characterize the behavioral consequences of alcohol-related brain damage of uncertain specificity.

We have proposed analyses appropriate for a dose–response model that posits or assumes a single factor, which we have called latent brain damage, underlying a wide range of child outcomes. In this context analysis entails a simultaneous nonlinear calibration of each of multiple measures of maternal alcohol consumption to form a composite measure of dose that best characterizes the prenatal alcohol exposure for the purposes of prediction of a battery of child outcomes. Outcomes measured at the same

age are treated symmetrically with dose in the calculation of a composite measure of behavioral deficit that best represents the brain damage predicted by prenatal alcohol exposure. This is the method of partial least squares analysis (PLS), as explained in Sampson et al. (66), Streissguth et al. (22), and Bookstein et al. (62), from which the following brief summary is derived. [Outcomes measured at different ages can be sorted by a longitudinal variant of this analysis (22).]

We assume multiple measures of dose or exposure, denoted X_1, \dots, X_m and multiple measures of response or outcome Y_1, \dots, Y_n , each scaled to variance one and assessed on N subjects. We then compute vectors of coefficients $\mathbf{A} = (A_1, \dots, A_m)$ and $\mathbf{B} = (B_1, \dots, B_n)$ that define composite dose and response scores or “latent variables” as $LV_X = \sum_{i=1}^m A_i X_i$ and $LV_Y = \sum_{j=1}^n B_j Y_j$ having the greatest covariance of any pair of such linear combinations for which the coefficient vectors \mathbf{A} and \mathbf{B} both have sum of squared coefficients equal to 1. The elements A_i of the vector \mathbf{A} are proportional to the correlations of the corresponding X -block variable X_i with the latent variable LV_Y representing the Y 's, and similarly, the elements B_j of the vector \mathbf{B} are proportional to the correlations of the corresponding Y -block variables Y_j with the latent variable LV_X representing the X 's. When it is known a priori that a construct that the X 's share causes changes in a construct that the Y 's share, these coefficients may be called saliences. Each A_i is the salience of the variable X_i for the latent variable representing the Y -block, and each B_j is the salience of the variable Y_j for the latent variable representing the X -block.

These coefficient or salience vectors can be computed using an iterative algorithm derived from their implicit characterization as correlations as just described, or it can be shown that this characterization leads equivalently to their computation as the first pair of singular vectors of the $m \times n$ correlation matrix \mathbf{R} between the dose and response scores. The latter suggests further interpretations of the saliences as explanations of the pattern of correlations between the measures of dose and response. For further discussion see Streissguth et al. (22) or Bookstein et al. (62).

The calculation of saliences and latent variable scores must also recognize that our multiple alcohol measures arise on a variety of separate scales—ounces per day, counts of various sorts, categorizations of bingeing behavior, and simple dichotomies. For all of these except the last, conventional methods of nonlinear scaling apply to linearize the dose scale with respect to the composite outcome. (We choose to rescale or linearize only the dose measures, not the neuropsychological

outcomes.) This linearization is computed using now-common scatterplot smoothers nested within the iterative algorithm for the calculation of the saliences as correlations. Following this iterative calculation, one of the basic summaries of the analysis is then a simple scatterplot of the scores of the Alcohol and Outcome latent variables, LV_X and LV_Y , as illustrated in Figure 5 based on the data presented in Sampson et al. (67).

This approach is particularly useful for assessment of the form of the dose–response relation. The issue of whether there are meaningful thresholds requires consideration both of the scatterplot of latent variable scores illustrated in Figure 5 and of (smoothed) dose–response scatters for the composite latent variable outcome score against each of the single dose measures. The PLS LV is a combination of alcohol scores that is monotone increasing in each of its components and that is low or zero only when all components are low or zero. The group of subjects with low/zero scores on the alcohol LV is the most meaningful definition of a subgroup having no average alcohol-related deficits on the corresponding outcome LV. The definition of this group in terms of the nonlinear transformations of each of the individual alcohol components will be the basis of any conclusion about thresholds.

The PLS analysis underlying Figure 5 summarizes the relationship between 13 measures of prenatal alcohol exposure from maternal self-report and 25 outcome scores describing performance on three different cognitive tasks for 368 fourteen-year-old offspring of nonabstaining mothers (67). The scatterplot smoother drawn in Figure 5,

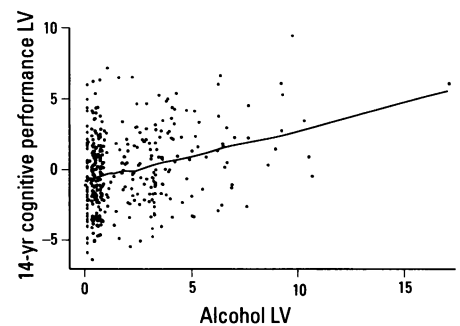


Figure 5. Scatterplot of scores on the first Latent Variable pair based on offspring of nonabstainers from Sampson et al. (67). The horizontal axis represents scores on the alcohol latent variable computed as a combination of 13 nonlinearly transformed measures of prenatal alcohol exposure reflecting different aspects of volume, pattern, and timing of maternal consumption. The vertical axis represents scores on an outcome latent variable computed as a composite from 25 measures of cognitive performance from three tasks assessed at 14 years of age. The structure of the trend indicated by the scatterplot smooth is not affected by deletion of the one extreme point.

which begins at the smallest nonzero dose, clearly provides no evidence of any threshold. Furthermore, a meaningful biological threshold can only be claimed if thresholds are apparent in the (nonlinear) relationships of each of the components with the composite outcome LV. We have found considerable consistency in these nonlinear relationships across a diverse selection of outcomes. The individual measure of prenatal alcohol exposure with the most consistently high salience across seven published nonlinear PLS analyses was average drinks per occasion prior to recognition of pregnancy (ADOCCP). Figure 6 shows plots of the estimated relationships of this most salient dose measure against the outcome LV for each of these, again based only on nonabstainers. The standard deviations of the outcome LV scores have been scaled to variance one for these plots. The transformations are largely consistent and three out of seven are monotone down to the smallest nonzero scores (ADOCCP = 1.5; see Figure 6 caption). The transforms for the average daily volume measures [see (22,24,66–70) for examples] show the greatest instability across outcomes. If analysis had been conducted using only average daily volume scores, thresholds would have been suggested for many outcomes. Using the wrong dose measure is like using a unitary outcome measure, e.g., the face of FAS, in that it leads to misleading inferences about dose–response relations.

Discussion

In 1981 David W. Smith, one of those who identified and named fetal alcohol syndrome 8 years earlier, said:

One extremely important concept is to speak and to write of Fetal Alcohol Effects rather than Fetal Alcohol Syndrome. One finds every gradation from FAS to milder effects of alcohol on the developing fetus. Of greatest concern are the effects on brain development and function which include microcephaly, poor organization of brain, mental deficiency, behavioral aberration (especially hyperactivity) and neurological dysfunction (including “cerebral palsy”). (13)

This challenge has gone unanswered until quite recently, not for lack of sympathy but because it can be considered only by eschewing the “face of FAS” as a diagnostic standard in favor of joint measurement or calibration of brain development and function. Tools for this task have been developed only in the late 1990s. What we learned while we were awaiting these tools is just how poor a guide to alcohol-related damage is the face of FAS. In the middle range of neurological deficits (i.e., those milder than frank mental retardation) patients diagnosed FAS are, as a group, little different from those diagnosed with fetal alcohol effects in terms of behavior. As Smith implied, what is needed first is an objective calibration of alcohol-related brain

damage independent of the face. This goal can now be reached using new methods of multivariate neuroimage analysis and dose–response analysis. Only on the basis of this calibration can one properly assess the two principal issues addressed in this paper: the categorization of individuals, whether for the purpose of syndromology or for service-relevant diagnosis, and the possible categorization of exposure in terms of meaningful thresholds based on dose–response analysis of alcohol-related damage. It is a practical necessity that we sort exposed individuals by the extent of alcohol-related deficits. Thereafter, arriving at a cut-point for a categorization of those of greatest deficits is essentially a social process determined by political and economic constraints.

While the FAS diagnosis indubitably signals the fact of alcohol damage, as a severity score it is essentially social, not neuronal. It is not the answer to a question about this scientific calibration. James Harris writes in a chapter on testing in developmental neuropsychiatry (71):

Developmental neuropsychiatric assessment requires the concurrent measurement of cognitive, emotional, social, and global adaptive functions. Because problems in each of these areas may arise from brain dysfunction, . . . neuropsychological testing integrates psychiatric and psychological information on behavior and the mind with neurological information on the brain

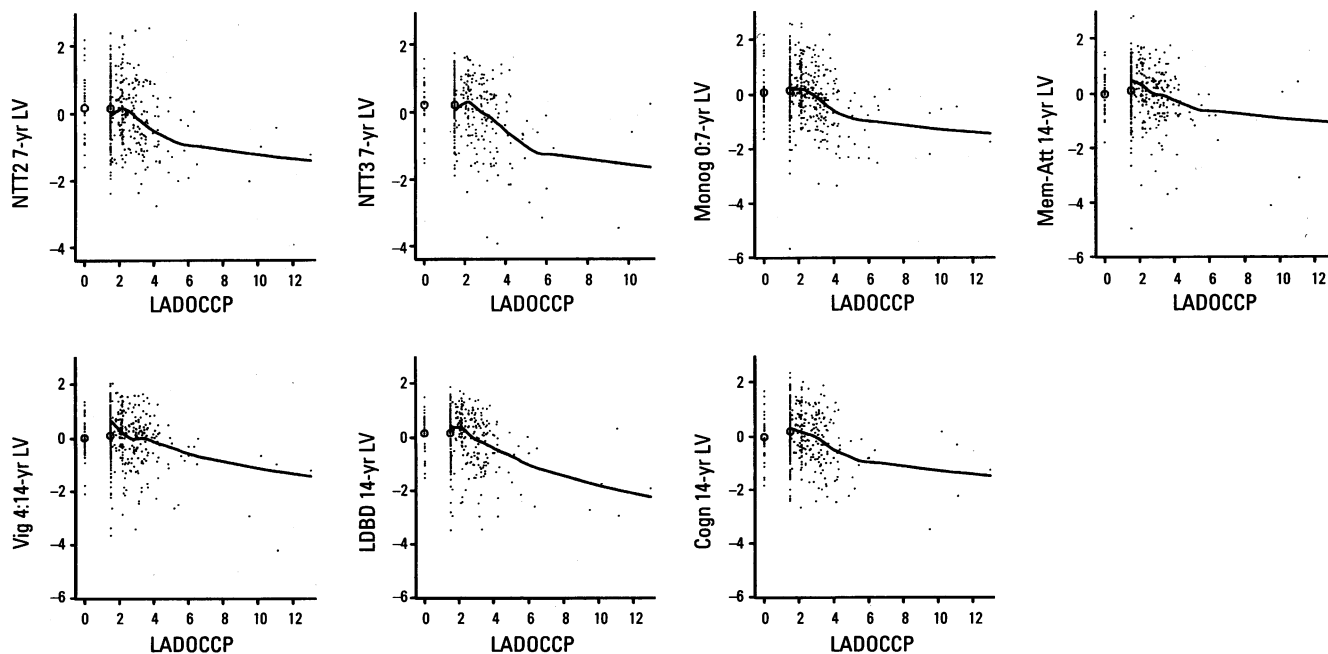


Figure 6. Nonlinear transformations of average drinks per drinking occasion prior to recognition of pregnancy (ADOCCP) for outcome latent variables based on data from seven published nonlinear PLS analyses: 7-year behavior/performance (66); 7-year neuropsych/neuromotor (68); 0–7 year composite (22); 14-year attention/memory (24); 4- to 14-year longitudinal vigilance (69); 14-year behavior and learning problems (70); and 14-year cognitive processing (67). These plots are based on analyses without the offspring of abstainers. The “loess” scatterplot smoother from Splus was applied to ADOCCP on a logarithmic scale for all the cases having scores greater than the minimum possible score, 1.5 (a coding for 1–2 drinks). Results are depicted here in original units of drinks, with means indicated by circles for the two subgroups of cases having scores zero (although nonzero on other measures of maternal consumption) and 1.5.

and nervous system.... The adult neuropsychological data base was largely established through the evaluation of adults with known brain damage.... The types of lesions seen in adults, such as strokes and penetrating wounds, occur far less commonly in children where congenital malformations related to pre and postnatal insults are more common. A majority of the neuropsychological dysfunctions of early life are not then associated with known brain insults, nor are they associated with lesions demonstrable on known neuroimaging studies.

From this point of view, the FAS face has been treated as a "lesion"—but it is not pertinent to answering Smith's question about fetal alcohol effects. Instead, we have proposed (72) a specific empirical interpretation of what is meant by integration of information on behavior with information on the brain: the search for paired patterns of sample variation, one pertaining to neuroanatomy and the other to profiles of behavior, that have highest covariance. This can be accomplished to a quite surprising extent by combining PLS (49) with established biometric methods for shape that we do not have the space to describe here (73). Findings to date (in a study of adult males) indicate no meaningful differences in brain-behavior relationships for those with/without the face—those diagnosed FAS versus FAE—and no evidence of any threshold of particularly severe damage that might lead to some convenient qualification for social services. Instead, there may be clinically relevant subtypes of fetal alcohol damage independent of both the face and net severity.

The recent Institute of Medicine (16) report proposed a 5-fold categorization including two categories: *a*) "partial FAS with confirmed maternal alcohol exposure" and *b*) "alcohol-related neurodevelopmental disorder (ARND)," intended to apply to patients who show particular behavioral or cognitive abnormalities or delays (e.g., learning difficulties, or poor metacognition) that "cannot be explained by familial background or environment alone." We argue elsewhere (49) that it is not fruitful to attempt identification of characteristics "that cannot be explained by ... environment alone." The task of assessing that alternative explanation not only is more difficult than the teratogenic assignment but distracts from the task of explaining the brain damage. Instead, the behavioral and cognitive abnormalities to be scrutinized should be those found linked to the crucial intervening variable for any behavioral teratology study, viz, the trace of prenatal brain damage revealed in analyses of neuroanatomic or neurochemical structure (as

by magnetic resonance imaging), neuropsychological performance or behavior, and prenatal alcohol exposure as best it can be assessed. The analytic strategies we have proposed calibrate explicitly and directly this link that connects the two components of the IOM specification of ARND: *a*) neurodevelopmental abnormalities, and *b*) behavior and cognitive abnormalities.

As argued above, the answer to the question of whether there are thresholds of exposure should be addressed taking note of biological mechanisms underlying threshold effects, carefully designed low-dose animal studies, and the most comprehensive (multivariate) calibration of alcohol-related brain damage in human studies. The latter provide our best representation of low-dose effects in humans. We do not claim that there is no threshold, but that on the basis of our data and plots like those in Figure 6, there is no evidence of a threshold. The question of low-dose teratogenesis is separate from the issue of qualification for services, of course; it is an essentially statistical issue but of huge import for guidelines and warnings.

We agree with Smith's belief (13) in a continuum from unaffected through various manifestations of fetal alcohol effects, including FAS. We also agree with Harris (71) that it is the consequences for brain functioning, not any "lesion-like" effects, that organize the most effective investigations. The statistical summaries of the two studies that provided the data reported here confirm the wisdom of both these clinicians. The heterogeneity of the fetal alcohol domain is not usefully studied either by categorization or by thresholding; it requires sensitive attention to a great variety of patterns and rhythms of dose and to profiles of neuroanatomical and neurobehavioral deficit.

As Holmes (74) wrote, the severe end of the spectrum of alcohol effects has been overemphasized; he encouraged focusing on the more subtle effects of alcohol on the fetus, those that can result in behavioral problems and cognitive dysfunction. The methodology now exists for doing this in a multidimensional manner respecting the complexity of dose-response relationships that involve many dimensions of dose, many dimensions of developmental outcome, and many ages of development. Inappropriate categorizations in alcohol teratogenesis obscure our understanding of the underlying dose-response mechanisms, and of the rich range of outcomes. The unfortunate focus of attention on only the severe end of the spectrum of alcohol-related deficits has unnecessarily complicated the provision of needed services to those falling outside the narrow bands of eligibility designed for other types of disabilities.

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