Tryptophan Contaminants Associated with Eosinophilia-Myalgia Syndrome

Rossanne M. Philen,1 Robert H. Hill, Jr.,2 W. Dana Flanders,1 Samuel P. Caudill,2 Larry Needham,2 Leeann Sewell,1 Eric J. Sampson,2 Henry Falk,1 Edwin M. Kilbourne,3 and the Eosinophilia-Myalgia Studies of Oregon, New York, and New Mexico

Eosinophilia-myalgia syndrome (EMS) has been linked to ingestion of tryptophan contaminated with 1,1′-ethylidenebis[L-tryptophan] (EBT), but other contaminants have received little study. The authors identified 101 lots of L-tryptophan that had been consumed either by persons with EMS or by asymptomatic tryptophan users and quantified the amounts of EBT and five other contaminants in each lot. After stratification of case and noncase lots by time of manufacture to adjust for the strong sequential pattern over time among case and noncase lots, higher EBT levels were still associated with a lot's case status, but the association lacked statistical significance (p = 0.120, odds ratio = 1.56, 95% confidence interval 0.758-3.23). While these findings do not rule out the possibility that EBT is the etiologic agent in EMS, they raise the possibility that other chemical contaminants in manufactured tryptophan modify the effects of EBT or that the causal agent of EMS is an entirely distinct compound. Am J Epidemiol 1993; 138:154-9.

eosinophilia; eosinophilia-myalgia syndrome; tryptophan

The eosinophilia-myalgia syndrome (EMS) epidemic has been associated with the consumption of L-tryptophan (1–8). Although L-tryptophan had been available for years, most persons developed EMS from July through October 1989. Previous work has linked EMS to ingestion of L-tryptophan contaminated with 1,1′-ethylidenebis[L-tryptophan], or EBT (also known as “peak 97” or “peak E”) (6, 9–11), but other potentially etiologic contaminants have received little study. Therefore, we designed a study to examine the relation between several L-tryptophan contaminants and EMS by comparing the amounts of six contaminants—EBT and peaks 98, 100, 200, 300, and 400—in lots associated with EMS (case lots) and lots not associated with the disease (noncase lots) in persons with limited, well-documented L-tryptophan exposure. The structures of peaks 100, 200, 300, and 400 have been determined and correspond to peaks 3, 4, 5, and 6 as reported by Müller and colleagues (12).
MATERIALS AND METHODS

Most persons who ingested \( \text{L-tryptophan} \) used several brands and switched brands frequently. Previous studies have linked the \( \text{L-tryptophan} \) produced by one manufacturer to responsibility for the epidemic (6, 7, and Dr. Lynn Miller, Centers for Disease Control and Prevention, personal communication, 1991). To concentrate on those brands and manufactured lots of \( \text{L-tryptophan} \) most likely to contain the etiologic agent of EMS, we restricted all analyses to lots made by the implicated manufacturer.

Case selection

We used information from a joint trace-back study conducted by the Centers for Disease Control and Prevention and the Food and Drug Administration (Dr. Lynn Miller, Centers for Disease Control and Prevention, personal communication, 1991). In this study, interviews of EMS patients were used to identify “priority” patients who developed EMS after brief exposure to a single brand of \( \text{L-tryptophan} \) (use of only one bottle of \( \text{L-tryptophan} \), or use of one brand for 60 days or less). All lots of \( \text{L-tryptophan} \) taken by one or more priority patients were considered potential case lots for our study.

In their joint study, researchers from the Centers for Disease Control and Prevention and the Food and Drug Administration identified 60 priority patients (Dr. Lynn Miller, Centers for Disease Control and Prevention, personal communication, 1991). Trace-back of samples obtained from these 60 patients established that the samples were produced from 74 lots of \( \text{L-tryptophan} \), 58 of which were from the implicated manufacturer. Data on chemical analyses of 52 of these 58 lots were available; these 52 lots were designated the case lots in our study.

We identified asymptomatic \( \text{L-tryptophan} \) users from information provided by the state health departments of New Mexico, New York, and Oregon (7), which had performed case-control studies of symptomatic and asymptomatic \( \text{L-tryptophan} \) users. To determine potential noncase lots, we compared the lot numbers of the \( \text{L-tryptophan} \) used by asymptomatic individuals in the states’ case-control studies with data on all lots reported to have been used by any patient with EMS in the Centers for Disease Control and Prevention/Food and Drug Administration study. All lots found in products consumed by patients with EMS were excluded from the noncase lot group. This left 49 noncase lots of \( \text{L-tryptophan} \) for which data from chemical analyses were available.

Chemical analysis

As part of previous and ongoing studies of the EMS epidemic, our laboratory had access to 412 samples from 322 lots of \( \text{L-tryptophan} \) produced and supplied by the implicated manufacturer from 1987 through 1989. High-performance liquid chromatography analysis of blind-coded samples quantitated amounts of several contaminants, including EBT (figure 1) and compounds arbitrarily designated as “peaks 98, 100, 200, 300, and 400” using a previously described method (13). We chose these peaks for study because they were among the largest of the contaminant peaks and because EBT had been studied previously.

![Figure 1](https://academic.oup.com/aje/article-abstract/138/3/154/86044/fig1)
Statistical analysis

We used the tryptophan lots (case or non-case) as the unit of analysis. To investigate the association of specific tryptophan contaminants with case and noncase lots, we first used several methods of exploratory data analysis, including graphs, univariate logistic regression, and the Wilcoxon rank sum test. For these analyses, we simplified by treating the observations as though they were independent of the time of lot manufacture. Because of strong serial autocorrelation in both the outcome and peak measurement variables, our definitive analysis of these data was conditional logistic regression that was based on lots grouped into sets according to date of manufacture. We adopted this approach because both the risk and levels of several contaminants increased during the epidemic period, making time behave as a confounder.

First, we ordered all case and noncase lots by production date, which ranged from August 19, 1986, through August 19, 1989. We then grouped lots by production dates without regard for contaminant level, using 2-month intervals beginning with August 1986. Ten groups contained only case or only noncase lots of L-tryptophan and could not be included in the analyses. The six time periods for which we had samples from at least one case lot and at least one noncase lot were November and December 1987, July and August 1988, November and December 1988, January and February 1989, March and April 1989, and July and August 1989.

We also used two other statistical methods designed for examining time-dependent events: 1) autoregressive logistic regression and 2) Box-Jenkins autoregressive integrated moving average modeling time series analyses, using health outcome as the independent variable and contaminant level as the dependent variable (14). With these methods, however, we did not account for the unequal time intervals between observations in our study. We therefore considered these to be supplementary analyses.

Univariate analyses and regressive logistic analyses were done using SAS PC version 6.1 (SAS Institute, Inc., Cary, NC). Conditional logistic regression was done using EGRET analysis module version 0.25.1 (Statistics and Epidemiology Research Corp., Seattle, WA). The Box-Jenkins autoregressive integrated moving average models were done on a mainframe computer with SAS PROC ARIMA (15, 16). We considered p values of 0.05 or smaller as statistically significant. Before analyses, we standardized peak measurements by dividing the relative response for each peak by the corresponding standard deviation among the noncase lots.

RESULTS

Analyses not controlling for time of manufacture

Our initial univariate analyses showed measurements of EBT and peak 200 to be significantly higher in case than in noncase lots; however, concentrations of peaks 98, 100, 300, and 400 were significantly lower in the case lots. Multiple logistic regression analyses done without including the time element showed that EBT was more strongly related to case lots than were the other contaminants. In addition, we found a strong negative association between case lots and peak 100 in multiple logistic regression analyses. The level of peak 100 was an important predictor of case or noncase status, even after the level of EBT was controlled for (p = 0.0001).

Analyses controlling for time of manufacture

In the conditional logistic regression matched on period of manufacture, EBT was still associated with case lots, but the association lacked statistical significance (p = 0.120, odds ratio = 1.56, 95 percent confidence interval 0.758–3.23). As in the univariate analyses, peak 98 was associated with lower risk, but not significantly so. Using the conditional logistic regression model, we found that other contaminants...
were only weakly associated with case or noncase status (table 1).

In regressive logistic analyses, the case or noncase status of lots that were 1 or 2 lags preceding any given lot strongly contributed to the logistic model \( (p = 0.0275) \). Although EBT continued to be associated with case lots in these models, the results lacked statistical significance \( (p = 0.0984) \), and no other contaminant was significantly associated with case or noncase status.

The case-noncase variable was not significantly associated with the contaminant series in any of the Box-Jenkins autoregressive integrated moving average models. Although the case-noncase variable remained the most strongly associated with EBT, the \( t \) ratio was only 1.13, not statistically significant.

### DISCUSSION

Our data confirm a previous observation that EBT levels in lots of L-tryptophan associated with EMS were higher than in other lots. However, as shown in figure 2, any variable that changed, perhaps even coincidentally, at the time EMS-associated tryptophan began to be produced would show an apparent link with illness. Therefore, to try

**TABLE 1.** Results of a conditional logistic regression model* in a study of contaminants in case- and noncase-associated lots of L-tryptophan in an outbreak of eosinophilia-myalgia syndrome: United States, 1989

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBT†</td>
<td>1.56</td>
<td>0.758–3.23</td>
</tr>
<tr>
<td>Peak‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>0.710</td>
<td>0.151–3.35</td>
</tr>
<tr>
<td>100</td>
<td>0.124</td>
<td>0.009–1.62</td>
</tr>
<tr>
<td>200</td>
<td>0.608</td>
<td>0.207–1.79</td>
</tr>
<tr>
<td>300</td>
<td>2.75</td>
<td>0.620–12.2</td>
</tr>
<tr>
<td>400</td>
<td>2.37</td>
<td>0.604–9.32</td>
</tr>
</tbody>
</table>

* This model stratifies by time period, and thus controls for the time of manufacture of L-tryptophan.
† 1,1'-ethylidenebis[L-tryptophan].
‡ Peaks 98, 100, 200, 300, and 400 are contaminants that have been found in L-tryptophan that has been associated with eosinophilia-myalgia syndrome. The chemical structures of peaks 100, 200, 300, and 400 have been identified by Müller et al. (12).

Our results do not vindicate EBT as a cause of the eosinophilia-myalgia syndrome epidemic. Misclassification of lots as case or noncase could have biased results toward the null. Moreover, the methods used to take into account the lack of independence among observations over time probably limited our power to detect associations. For example, when we stratified on time, only six strata contained both case and noncase observations, and most of the six strata included many case lots and few noncase lots, or vice-versa. Consequently, controlling for time reduced our power, and we may have failed to detect a real association. The low power reflects a real paucity of information
to identify which specific contaminant was responsible for EMS, we controlled for time of manufacture in our analyses.

In case-control studies, justification of statistical analyses usually requires that observations be independent. However, contaminant levels and the risk of EMS were both serially autocorrelated. Correlation of contaminant levels may reflect production processes in which lots of tryptophan could contain material from one or more fermentation batches of tryptophan; or conversely, one fermentation batch of tryptophan could form part of one or more production lots. Similarly, the risk of EMS appeared to rise during the epidemic period. Thus, these observations cannot be considered independent, providing additional justification for analyses that control for time.

In addition to conditional logistic regression, we used two other methods to account for the nonindependence of observations: regressive logistic regression and classic Box-Jenkins autoregressive integrated moving average modeling. These additional analyses were limited in part because we did not specifically model the unequal spacing of the manufacturing dates. Nevertheless, these two techniques yielded results that were virtually identical to results of our conditional logistic regression analysis. In all three analyses, EBT was positively but not significantly associated with case lots.

that could be used to determine whether the apparent association of EBT with illness is coincidental.

Our findings do show that the information available only weakly supports an association between risk and any one specific contaminant such as EBT, and they raise the possibility that other factors that changed concomitantly with EBT played a role. One possibility is that other chemical contaminants in certain lots of tryptophan may modify the effects of EBT. Since many trace contaminants have been found in case-associated lots of L-tryptophan (13), and this study only addresses six of these, other contaminants or interactions among several contaminants may account for the production of disease.

We also know that cases of EMS occurred for several years before the large epidemic in 1989, when EBT was not found in high levels in samples of L-tryptophan (4). We had information on L-tryptophan lots beginning in August 1986. We attempted to include the entire time for which we had information, and to account for the early cases of EMS in our analysis, in contrast to previous researchers, who included only epidemic cases (6). The finding of numerous trace contaminants and the occurrence of pre-epidemic cases of EMS further suggest that other factors may play a causal role, although we currently have insufficient data to identify specific etiologic agents for EMS.

The EMS experience suggests that other products intended for human consumption could become unintended vehicles for identical or similar toxicants in the future. Elucidation of the etiologic agent of EMS may lead to the identification of an entirely new
class of human toxicants and may help to explain the causes of clinically similar disorders of obscure etiology, such as scleroderma.

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REFERENCES