Lessons from a Danish Study on Neuropsychological Impairment Related to Lead Exposure

by Philippe Grandjean,* Troels Lyngbye,* and Ole Nørby Hansen†

Serious problems emerge when evaluating evidence on lead neurotoxicity in children. The extent of these problems and ways to control them were explored in a study of 1291 children from the first class in the schools of Aarhus municipality, Denmark. The lead retention in circumpulpal dentin in shed deciduous teeth was used as an indicator of cumulated lead exposure; it correlated most strongly with traffic density at the residence of each family and at the day-care institutions. In a nested case-control group selected on the basis of dentin lead concentrations, 29 of 200 children had encountered obstetrical complications and other medical risks for neurobehavioral dysfunction; these children primarily belonged to the low-lead group. As lead-related neurobehavioral effects are nonspecific, inclusion of these children in the data analysis would therefore have distorted the results toward the null hypothesis. Children from the high-lead group who had experienced neonatal jaundice showed impaired performance when compared to other high-lead children; this finding may suggest a synergistic effect. The Bender gestalt test scored by the Göttingen system was the test that was most sensitive to lead exposure. The conclusion that neurobehavioral effects can be caused by the relatively low lead exposures in Denmark may not be surprising, as current exposures to this toxic metal greatly exceed the prepollution levels to which the human body originally adapted.

Introduction

Neurotoxic effects due to inorganic lead have been well documented in both experimental and epidemiological studies (1-3). However, unresolved, thorny questions remain, e.g., concerning the approximate threshold for such effects in humans and the contribution by lead exposure to overall neurobehavioral impairment occurring in modern society (Table 1). These questions are of key relevance in determining the degree of pollution abatement needed.

We have recently completed a neurobehavioral study of Danish children with a lead exposure that is lower than reported in studies from other countries. Our research strategy has taken into account as far as possible the problems indicated in Table 1. Thus, the evidence from our study may offer a contribution to resolving the above concerns.

The background population of children included all of those attending the first class in the schools of Aarhus municipality, Denmark, in 1982 to 1983, a total of 2412 eligible children. Within this cohort, we selected a nested case-referent group of 200 children who were examined in detail (4).

Table 1. Weaknesses of current evidence on lead neurotoxicity in children and possible epidemiological strategies to minimize these limitations.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Approach</th>
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<tbody>
<tr>
<td>Irregular exposure</td>
<td>Chronic exposure assessment</td>
</tr>
<tr>
<td>Variable absorption</td>
<td>Cumulated dose measurement</td>
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<tr>
<td>Nonspecific effects</td>
<td>Confounder analysis</td>
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<td>Individual susceptibility</td>
<td>Modifier analysis</td>
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<td>Elusive threshold</td>
<td>Groups with minimal exposures</td>
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<td>Compensation and repair</td>
<td>Long-term follow-up</td>
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Lead Exposure and Absorption

Preventive efforts must be aimed at minimizing or eliminating exposure sources that contribute to the adverse effects. Ideally, documentation should then be provided to enable a ranking of the individual emission sources with regard to their toxic potentials. However, lead exposure in children is difficult to characterize because of the multitude of individual sources and pathways and the variability of exposures over time. Furthermore, the bioavailability of inhaled or ingested lead from different sources may vary considerably. Consequently, any attempt to characterize in any detail the relation between individual types of lead exposure in society and their contribution to adverse health effects is bound to fail.

Accordingly, epidemiological studies in lead neurotoxicity have used parameters indicating lead absorption, primarily the lead concentration in blood and/or teeth (1-3). We chose to...
determine the lead content of circumpulpal (or secondary) dentin from shed deciduous incisors (3,6). This tissue is formed very slowly after the eruption the tooth; the dentin has a blood supply that permits continuous ion exchange and retention of lead as lead phosphate. Experimental evidence shows a dose-response relationship between blood lead and dentin lead (7). Also, increasing lead concentrations have been documented in secondary dentin of permanent teeth with increasing age (8,9) and in deciduous teeth with augmented exposures (10,11). Thus, lead retention in secondary dentin appears to provide an index of cumulated lead absorption during the period of active blood supply of the tissue, i.e., from the time of tooth eruption to tooth shedding. This period of life coincides with the age at which the developing nervous system of the child is particularly sensitive to adverse effects. Also, as the half-life of lead retained in the brain may be substantial, a measure of the cumulated absorption may also reflect the retention of lead in the central nervous system.

Two different methods of dentin preparation have been used in the past. Shapiro et al. (12) chipped off the secondary dentin from a 600-μm cross-section of the tooth, and this method was used by Needleman et al. (13) and in a New Zealand study (14). We used a wolfram carbide rosette burr to separate the dentin from the tooth (5). Several other studies [reviewed in Grant et al. (1), and Smith (2)] have used whole teeth or tooth crowns; such samples contain different proportions of enamel and primary dentin that probably reflect lead absorption levels to varying degrees. Not surprisingly, the lead concentration in the tooth as a whole correlates poorly with the level in circumpulpal dentin, the average lead concentration of the latter being about five times greater (6). That the lead concentration in dentin is an important predictor is stressed by the fact that two of the studies that showed the most marked lead-related decreases in neurobehavioral performance (4,13) were based on dentin measurements.

As chronic lead exposure levels are difficult to characterize by questionnaire or monitoring data, only a few major exposure indicators would be expected to correlate significantly with the dentin lead. Most importantly, dentin lead was associated with the traffic density at the residence of the children at ages 0.5 to 3 years (15). This correlation was based on municipal traffic counts at all addresses where the children had resided. Also, traffic counts from day-care institutions were obtained, and a 1:1 ratio between traffic counts at home and at the day-care institution resulted in an improved correlation with dentin lead (15). Further, parents of children with a high dentin lead concentration were automechanics and shipyard workers more frequently than expected, thus suggesting a contribution from indirect occupational lead exposure (15). These associations are entirely plausible and therefore lend support to the notion that dentin lead is a useful indicator of chronic exposures.

The average lead concentration varied somewhat with the tooth type, but most averages were close to 10 μg/g (0.05 μmole/g). The distribution was skewed (Fig. 1). Children for detailed examination were identified on the basis of their dentin lead concentrations. The case group consisted of the 110 children with a dentin lead ≥ 18.7 μg/g (0.090 μmole/g) or, if two or more teeth were analyzed, an arithmetic mean ≥ 16.0 μg/g (0.078 μmole/g). This case group was then matched to control children among those with concentrations below 5 μg/g (0.024 μmole/g). The matching was performed for gender and parental socioeconomic status; a total of 103 control children were identified.

Blood for lead analysis was obtained from most of these children when they were 9 to 10 years old (Fig. 2) (16). Although the dentin lead concentrations differed considerably between the two groups, the blood lead levels were quite similar. The children with the highest dentin lead levels had blood lead concentrations of 0.08 to 0.63 μmole/L (16–130 μg/L) and a geometric mean of 0.28 μmole/L (58 μg/L). In comparison, the children with the lowest dentin levels had blood lead concentrations of 0.08 to 0.70 μmole/L (16–144 μg/L) and a geometric mean of 0.18 μmole/L (37 μg/L). Thus, the different lead burdens earlier in life were only poorly reflected in the lead concentrations in blood.

**Nonspecificity and Individual Susceptibility**

Early effects of lead toxicity are nonspecific, lead being one out of several factors influencing neurobehavioral performance.
Thus, normal neurobehavioral development in children will depend upon genetic factors as well as environmental stimuli. In addition, a range of risk factors may suppress or delay the development of the nervous system in children (17). The presence of one or more of such risk factors could potentially render a child more susceptible to the effects of lead. These concerns must be taken into consideration in the design, statistical analysis, and evaluation of a study.

Much emphasis has been placed on maternal intelligence as a potential confounder (2). Although the precision of a study may be increased by determining the mother’s IQ, this factor may not necessarily behave as a confounder, unless the lead exposure of the child depends on maternal intelligence. As many women were expected to withdraw from a study requiring IQ testing, we decided to determine only the duration of maternal education. The educational score explained some of the variance of the children’s IQ, but it did not confound the association with lead retention (4).

In the total cohort, dentin lead showed no relation to socioeconomic class. In this regard, the situation in Aarhus municipality may differ in a fortunate way from other settings, e.g., the U.S., where increased lead exposure is likely to occur in delapidated inner-city neighborhoods (18). In addition, we found that the effects of lead on neurobehavioral performance were similar in all socioeconomic strata of the case-control group (4).

As neurobehavioral performance may be impaired by other risk factors (e.g., obstetrical complications), we attempted to reduce the type-I error by restricting for all medical risk factors in the analysis of the data (17). Obviously, the classification and analysis were performed without knowledge concerning the lead exposure level. In restricting the analysis to children without any risk factors other than lead, our study differs considerably from other studies (1,2).

An important finding was that several well-known risk factors were associated with low dentin-lead. In addition, delayed motor development was also seen in these children (Fig. 3), thus providing a possible explanation for the lower lead exposures. Consequently, inclusion of these children in the data analysis would have distorted the results toward the null hypothesis (17).

Although most of the children with medical risk factors were in the low-lead group, a conspicuous exception to this pattern was seen with the cases of asphyxial episodes, i.e., near-miss sudden infant death syndrome (SIDS), that occurred only in three children from the high-lead group (19). Although lead could conceivably be a factor contributing to the development of SIDS, none of these children were included in the final data analysis. A total of 29 children were then disregarded because of low birth weight, obstetrical complications, neurological abnormalities, sensory defects, or near-miss SIDS (17).

With regard to neonatal jaundice, we applied current diagnostic criteria to eliminate only those children who had a high serum bilirubin above 280 μmole/L at 1 week of age (17). Less serious jaundice occurred more frequently in the low-lead group; the neurobehavioral performance of these children was similar to that seen in the control group in general. However, in the high-lead group, children with neonatal icterus (below the exclusion criterion exhibited neurobehavioral results that were significantly poorer than those of the rest of the case group (17). This finding would suggest a possible synergic effect between neonatal jaundice and lead exposure. Although the neurobehavioral significance of a limited degree of jaundice may be questioned, a recent publication reported that such physiological jaundice in connection with premature birth is associated with an increased risk of neurological abnormalities in early life (20).

Several neurobehavioral tests were employed to determine the character and extent of possible neurobehavioral dysfunction (4). The verbal Wechsler Intelligence Scale for Children (WISC) and the Bender gestalt test, as scored by the Göttingen system, showed significantly decreased performance in the children in the high dentin-lead group (Table 2).

As this difference could potentially be due to factors other than lead exposure, we conducted a detailed statistical evaluation, taking into account extensive demographic, questionnaire, and monitoring data. In the confounder analysis, we accepted all associations at a probability level of $p < 0.2$, and these associations were identified both by bivariate and multivariate analyses, the latter being the most efficient procedure. In the final statistical analysis, stepwise multivariate analysis was employed when the dependent variable was continuous, and logistic

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**Table 2. Number of errors on the Bender gestalt test, scored by the Göttingen system, and verbal IQ determined by WISC in pairs of children, matched by gender and stratified according to parental socioeconomic status (SES), in relation to high versus low lead concentrations in the dentin of shed deciduous teeth (4).**

<table>
<thead>
<tr>
<th>SES</th>
<th>Number of matched pairs</th>
<th>Low-lead mean</th>
<th>High-lead mean</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bender gestalt test, number of errors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>15</td>
<td>14.87</td>
<td>20.27</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>15.80</td>
<td>21.55</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IV</td>
<td>28</td>
<td>17.57</td>
<td>22.39</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>V</td>
<td>15</td>
<td>17.27</td>
<td>21.33</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>WISC, verbal IQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>15</td>
<td>119.73</td>
<td>106.47</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>113.55</td>
<td>102.55</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IV</td>
<td>28</td>
<td>106.36</td>
<td>101.07</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>V</td>
<td>15</td>
<td>108.40</td>
<td>103.60</td>
<td>&lt; 0.20</td>
</tr>
</tbody>
</table>
stepwise multiple regression technique was used when the dependent variable was of a categoric nature. The analyses were performed with the lead variable either as the first or the last step. Multivariate analysis showed that, of the 42% of the variance explained for the Bender gestalt test, lead exposure accounted for 29%; with the verbal WISC, lead explained 5% of the variance out of the total of 24% accounted for (4). The WISC test has been used in several other studies that have revealed similar relations to lead exposures at higher levels (2,3).

Existence of a Threshold

When considering the possible existence of a threshold for lead-induced neurotoxicity, the sensitivity and validity of the neurobehavioral tests employed must be scrutinized. This consideration must also take into regard the possible long-term health significance of subtle changes in the light of possible reversibility and mental capacity for compensation. Also, the range of lead exposures examined must be evaluated critically.

The Bender gestalt test, as scored by the Göttingen system, appeared to be the test that was most sensitive to lead exposure (4). This test determines form recognition and reproduction as well as fine motor coordination. It is one of the key components of modern neuropsychological batteries for examination of children. The Bender test has recently been evaluated in a case-control study of children with known early brain damage; of all neuropsychological tests employed, the Bender test was the one that provided the best discrimination between children with and without prior brain damage (21).

Although decreased test performance may not necessarily have severe implications for neurobehavioral function in general, we found an association with poor results in school. Thus, the high-lead group also had a higher proportion of children needing special education in school (22). This result would tend to confirm the significance of the test results. Again in this case, children with medical risk factors more frequently required special education, despite their low dentin lead levels; this confounding effect was controlled by restriction in the analysis (22).

The nervous system has a considerable capacity to compensate for minor injuries, and a toxic exposure may not necessarily result in any clinically detectable change. However, recent evidence has suggested that this reserve capacity is not constant through life but may decrease significantly with age (Fig. 4). This decline in the capability to compensate may then unmask the damages incurred previously (23). Because of such considerations, functional capacity of the nervous system must be regarded as an irreplaceable resource that should be protected against harmful exposures. The documented signs of neurotoxicity due to lead must therefore be regarded as a serious warning.

With regard to the exposure levels, the blood lead concentration was quite low both in the low-lead and the high-lead group, none of the children having lead levels above 0.70 μmole/L (144 μg/L) (Fig. 2). All blood lead levels were therefore below 150 μg/L (0.73 μmole/L), a level currently used in the U.S. as the limit for the upper "safe" lead exposure. The dentin analyses also documented that the Aarhus children had relative low lead concentrations in their body. For comparison, deciduous teeth from U.S. inner-city children may contain averages of about 100 μg/g (C. B. Ernhart, personal communication), i.e., about 10-fold higher than in Denmark.

On the other hand, prehistoric teeth from adults contain much lower lead concentrations at about 1 μg/g, with current Danish lead levels in adults being about 30-fold higher (9). Thus, the Danish children do not represent a true low-level exposure situation. The fact that neurobehavioral changes can be identified at current exposure levels may well be interpreted in the light of the considerable difference from prehistoric levels that reflect the environmental conditions to which the human body originally adapted.

Conclusions

The obstacles indicated in Table 1 have caused serious difficulties for epidemiological research in this area. The study performed in Aarhus has elucidated some of the research problems and explored ways to control them. Thus, although cross-sectional studies have particular limitations, the lead concentration in circumpulpal dentin of shed deciduous teeth has proven to be an effective indicator of cumulated lead absorption. With regard to confounding factors, medical risks for delayed neurological maturation will tend to limit the risk of increased lead exposures. Inclusion of such children in a study will therefore distort the results toward the null hypothesis. The conclusion that neurobehavioral effects can be caused by the relatively low lead exposures in Denmark may not be surprising, as current exposures to this toxic metal greatly exceed the prepollution levels to which the human body originally adapted. Thus, a threshold for neurobehavioral effects of lead may well be much below a blood-lead concentration of 150 μg/L currently stated as safe in the U.S.

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REFERENCES