following internationally suggested guidelines. Cases with chromosomal or single gene disorders, or with well-known dysmorphic syndromes, were excluded from further analysis. The distribution by type of LRD of the remaining 410 cases with an unknown cause are shown in the table. We then grouped the hospitals of the coastal areas and compared their rates of LRD with all remaining hospitals.

As shown in the table, LRD rates in coastal hospitals were not higher than in non-coastal hospitals, as evidenced by a rate ratio (RR) close to unity; this finding held for the total group of LRD as well as for individual types of LRD (types with 2 cases or fewer were not tested). No significant differences of LRD rates were seen between the eastern and western coast or between individual coastal provinces.

To investigate further the role of parental exposure to sea-related activities on LRD in offspring, we conducted a case-control study with the same IPIMC registry data for which we had information on parental occupation. Cases were defined as having a LRD, and controls as having an isolated minor anomaly such as a preauricular tag or small skin marks; a positive exposure was defined as having at least one parent in sea-related occupations (eg, fisherman or fish-seller).

Cases with LRD were not more likely than controls to have a parent employed in sea-related activities; only 4 of 397 (1%) cases had a positive exposure compared with 7 of 2108 (0.3%) controls (odds ratio 3.05; 95% CI 0.65–12.08). Of the 4 exposed cases, 2 had a postaxial defect (absence of fibula and fifth metatarsal bones; absent ulna and 3, 4, 5 metacarpals and digits); 1 had an intercalary defect (femur hypoplasia); and 1 had a complex defect of the multiple type (bilateral superior amelia, sacral schisis, femur hypoplasia, and absent fibula bilaterally).

Our study did not find evidence in Italy of an increased risk for LRD linked to births in coastal areas or to a sea-related parental occupation. Although caution must be exercised in the interpretation of these findings, because proxy measures for sea-related exposures were used, it is reassuring that no clusters association suspected because of a cluster must be independent

### Dose standardisation of botulinum toxin

Sir—The increasing clinical use of botulinum toxin calls for precise dose standardisation if this agent is to be used safely. Quinn and Hallet have aired this important issue and the need to standardise the biological unit of botulinum toxin activity for the American and British products. The number of mouse LD₅₀ units of toxin required for the treatment of torticollis in the UK is 875–1200 IU (Dysport) whereas in the US it is 100–280 IU (Botox), and the dose requirements for the treatment of essential blepharospasm differ similarly. Because there are also significant differences in the specific activities (mouse LD₅₀ units/ng) of these two preparations, Schantz and Johnson correctly point out that it is meaningless to express the dose of toxin in terms of mg (ng); and they also call for greater standardisation of the mouse LD₅₀ bioassay.

In our laboratory mouse LD₅₀ assays on a vial of Botox and of Dysport revealed a 1.9-fold discrepancy but that would not explain the 4.4-fold difference identified by Quinn and Hallet. An assay for botulinum toxin activity based on regional chemodenervation was devised to stimulate more closely the biological effects of the agent that are clinically important. With this assay an additional 2.4-fold difference was detected. 1.9 x 2.4 is 4.6, thus explaining the differences cited by Quinn and Hallet.

Although a standardised mouse LD₅₀ assay did reveal very significant differences in the units of activity defined for the two preparations, this method was insensitive to additional differences detected with regional chemodenervation. We agree that standardisation of the animal-based test is critical but the mouse LD₅₀ assay may not be good enough for the accurate preclinical characterisation of the clinical potency of botulinum toxin.

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### L-tryptophan and eosinophilia-myalgia syndrome

Sir—In July, 1993, I was asked by lawyers representing Showa Denko KK, a manufacturer of L-tryptophan, to review the epidemiological studies of the association of that product with eosinophilia-myalgia syndrome (EMS). The first epidemiological investigation was carried out in New Mexico soon after the State health authorities received 3 case-reports of an unusual disease characterised by severe eosinophilia, all 3 cases had used L-tryptophan. In a case-control study of 11 cases identified from a review of 125,000 haematological records, and of 22 matched neighbourhood controls, the numbers exposed to L-tryptophan were 11 and 2, respectively (p < 0.00002). Eidson et al concluded that this was a “causal association”.

The paper states that “some of the patients were reported by their physicians before our review of the eosinophil counts” but they were included as cases if they were “picked up independently during the review”, and in an acknowledgment the authors thank a physician “for notification of the cluster”. Eidson et al seem to have included an already identified cluster of exposed cases in a case-control study. A test for an association suspected because of a cluster must be independent