Standardization of an allergenic extract implies a quantitative comparison to a standard reference extract. Skin testing is the most commonly used in-vivo technique for making these comparisons. When the reference and test extracts are obtained from similar sources and tested in the same subjects, the relative potency of the test extract (% reference) can be precisely and accurately determined by parallel line bioassay using best-fit dose response lines employing intradermal erythema (1). Non-parallelism in this assay demonstrates to some extent that the extracts may be compositionally different. Parallelism on the other hand, insures that the relative potency is consistent regardless at which allergic response the potency is determined but does not assure compositional identity. A consistent relative potency enhances the safe switching of the test extract with respect to the reference based on their relative potency.

When extracts from diverse allergen sources (pollens, molds, etc.) are to be quantitatively compared by skin test, these extracts are known a priori to be compositionally different. They cannot be compared in the same subject because a single subject will not have similar sensitivity to each allergen source. Furthermore these products are not intended to be switched in the same patient, thus parallel line bioassay is not indicated for these comparisons. In this circumstance, Voorhorst suggested (2) that each extract be individually skin tested in populations of patients who have a high degree of skin sensitivity to their respective allergens. In this model, the dose of each extract required to give a specified skin reaction can be determined for each patient. The mean dose of extract required to give identical skin reactions can therefore be determined in each population and can be used as a measure of the biological activity of
each extract tested regardless of their source. A group of the most biologically active extracts in this model can then be designated as the reference group and the relative biological activity of any other extract can be computed in relation to that reference group based on the difference in mean (log) dilution required for the test extract with respect to the reference group to produce the identical skin response in their respective populations.

This model has been implemented as follows: Patients who were puncture tested (Wyeth bifurcated needle) with an allergen concentrate (manufacturer’s highest concentration sold for clinical use, e.g. 1:10 w/v) of either cat or short ragweed and have sum of erythema diameters ≥ 40 mm have been found to require at least a 10,000 fold dilution of the concentrate for an intradermal sum of erythema diameter = 50 mm (3). Based on these data, puncture test positive patients by the above criteria were considered highly sensitive to their respective allergen concentrates, regardless of allergen source, and were titrated to determine the intradermal dilution required to produce a 50 mm sum of erythema diameter response (D50) to their respective allergen concentrate. The D50 defined the allergenic activity of each extract in each patient. If the allergen concentrate was not potent enough to produce a positive puncture test by the above criteria, intradermal titrations could still be carried out.

PATIENT J.A.
SHORT RAGWEED

SLOPE = -30.6
r = -0.98

Intercept = 402.4

\[ D_{50} = \frac{50 - 402.4}{-30.6} \]

\[ D_{50} = 11.5 \]

Table 1: Mean D50

<table>
<thead>
<tr>
<th>Pollen</th>
<th>Weeds</th>
<th>Grasses</th>
<th>Trees</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When Y = 50 mm; \( X (D_{50}) = (50 - \text{Intercept})/\text{slope.} \)
Serial 3-fold dilutions of allergen, injected (0.05 ml) in singlicate and outlined at 15 minutes are used to generate the best-fit line using the first dilution where sum of erythema diameters ($\Sigma E$) is absent or approximates the wheal and the next 3 more concentrated serial dilutions giving a graded erythema response. Best-fit lines are valid if erythema response is monotonic, correlation coefficient $\geq 0.85$, and slope $\geq 10$ ($\Sigma E$ spans a wide range of values, e.g. 0 to 100 mm and brackets $\Sigma E = 50$ mm). The $D_{50}$ can be computed from the best-fit line (Fig. 1). The intradermal technique using erythema is preferred to puncture testing because it produces steeper dose-response lines (1) and is much more sensitive (5) requiring 1,000–10,000 lower doses of allergen for equivalent skin responses (1, 5), thus allowing evaluation of all types of reference extracts including low potency extracts which may not produce positive puncture tests.

The 2SD limits of the within-subject reproducibility of $D_{50}$ (log base 3) is $\pm 0.62$ for one patient (6). The 2SD limits for the between year, between locale and between investigator reproducibility of mean $D_{50}$ (log base 3) of similar extracts tested in different populations of highly sensitive patients is $\pm 1.06$ if 15 patients are tested for each extract. Extracts which fall outside these limits can be considered significantly different ($p < 0.05$) from each other in their in-vivo skin test activity.

The mean $D_{50}$ of standardized extracts from diverse sources were determined and grouped by allergen type (Table 1). Despite differences in allergen source and manufacture, a large number of allergen types had similar Mean $D_{50}$, suggesting that most allergen references could be prepared to have similar in-vivo activity if a specific mean $D_{50}$ was targeted for the final product. One scheme for targetting a mean $D_{50}$ is based on the frequency distribution of Mean $D_{50}$ of all the extracts tested (Fig. 2). The products with mean $D_{50}$'s

<table>
<thead>
<tr>
<th>Allergen Type</th>
<th># Exacts Tested</th>
<th>Mean $D_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Pollen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weeds</td>
<td>13</td>
<td>13.3</td>
</tr>
<tr>
<td>Grasses</td>
<td>9</td>
<td>14.2</td>
</tr>
<tr>
<td>Trees</td>
<td>5</td>
<td>11.8</td>
</tr>
<tr>
<td>B. Animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>3*</td>
<td>12.9</td>
</tr>
<tr>
<td>C. Insects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mite</td>
<td>2</td>
<td>13.5</td>
</tr>
<tr>
<td>Cockroach</td>
<td>2</td>
<td>12.0</td>
</tr>
<tr>
<td>D. Molds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternaria</td>
<td>3</td>
<td>12.0</td>
</tr>
</tbody>
</table>

*Mean $D_{50} = 12.1, 13.2, 13.4$. The latter two Mean $D_{50}$ were obtained using 10 fold dilutions, rather than 3 fold dilutions – a less accurate and precise method.
THE ID\textsubscript{50} EAL METHOD
(Intradermal Dilution for 50mm sum of Erythema diameters determines the Allergy units)

![Diagram showing frequency distribution of the Mean D\textsubscript{50}'s of 40 allergenic extracts. One hundred thousand Allergy Units/ml were assigned to the modal D\textsubscript{50} ± 2SD, i.e. 14 ± 1 (log\textsubscript{3}) dilution).](image)

SERIAL 3-FOLD DILUTION NUMBER FOR SUM OF ERYTHEMA = 50 mm (D\textsubscript{50})

with the highest frequencies were found between Dilution Nos. 13 to 15 (3\textsuperscript{-13} to 3\textsuperscript{-15} dilutions of their concentrate for 50 mm sum of erythema). Extracts in this Mean D\textsubscript{50} range include short ragweed and grass pollen extracts (Table 1). Extracts with the highest frequency D\textsubscript{50} were considered the reference group of extracts since they included many of the best studied and most biologically active extracts tested. This group was assigned 100,000 allergy units/ml. Since the Mean D\textsubscript{50} is the dose of allergen required for a given skin response in a population of patients, differences in Mean D\textsubscript{50} can be considered differences in the dose of allergen required for the identical in-vivo response regardless of allergen source and thus a population measure of the in-vivo biological activity of extracts. Thus, any candidate reference extract regardless of source can be assigned an allergy unitage based on its Mean D\textsubscript{50} using the scheme in Fig. 2 (ID\textsubscript{50} EAL). Once a reference is calibrated based on this scheme and assigned an allergy unitage, the relative potency of any test extract obtained from the same source can be assigned an allergy unit with respect to that reference by an assay of relative potency e.g. parallel line bioassay (1) or by an in-vitro test of potency, e.g. RAST inhibition.

If this method of biological standardization is to be truly valuable, then extracts obtained from diverse sources, which have identical Mean D\textsubscript{50} in

References
1. TURKELTAUB, P.
A standardized assay for airborne allergens by in vitro test on assay results
2. VOORHOST, R.,
Assessment of dust mite. Staff
3. Unpublished data
4. TURKELTAUB, C.
Evaluation of S. allergenic Prod
5. BELIN, L. and N
zited to B. sub
6. RASTOGI, S.C.,
of evaluating st
7. TURKELTAUB, P.
dose-dependent (part 2): 191, 1

Their respective administered in similar D\textsubscript{50}'s. The best method of biological standardization is to be truly valuable, then extracts obtained from diverse sources, which have identical Mean D\textsubscript{50} in
their respective populations should produce similar clinical responses when administered in identical doses (by the ID\textsubscript{50} EAL method) to patients with similar D\textsubscript{50}s. This has been demonstrated for the ID\textsubscript{50} EAL method (7) for rye grass and short ragweed, thus validating the clinical applicability of this method of biological standardization.

References

3. Unpublished data.